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COLLEGE OF AMERICAN PATHOLOGISTS CONFERENCE XVI
AIDS AND THE PATHOLOGIST
AUGUST 9-11, 1989

Conference Editor: Herbert F. Polesky, MD

233 **College of American Pathologists Conference XVI on AIDS and the Pathologist: Foreword**
Herbert F. Polesky, MD, Minneapolis, Minn

234 **Invited Participants and Guests**

235 **The Pathobiology of HIV Infection: A Review**
Roger D. Smith, MD, Cincinnati, Ohio

240 **The Western Immunoblot Procedure for HIV Antibodies and Its Interpretation**
Roger Y. Dodd, PhD,
Chyang T. Fang, PhD, Rockville, Md

246 **Immunofluorescence for Serodiagnosis of Retrovirus Infection**
Michael S. Ascher, MD,
Judith C. Wilber, PhD, Berkeley, Calif

249 **Human Immunodeficiency Virus Type 1 Antigen and Culture Assays**
J. Brooks Jackson, MD, Cleveland, Ohio

254 **Synthetic Peptide Assays to Detect Human Immunodeficiency Virus Types 1 and 2 in Seropositive Individuals**
Richard S. Smith, PhD,
D. Elliot Parks, PhD, San Diego, Calif

259 **Detection of Human Immunodeficiency Viruses by the Polymerase Chain Reaction**
John J. Sninsky, PhD,
Shirley Kwok, Emeryville, Calif

263 **Centers for Disease Control Perspective on Quality Assurance for Human Immunodeficiency Virus Type 1 Antibody Testing: Model Performance Evaluation Program**
Ronald O. Valdiserri, MD, MPH; Roger N. Taylor, PhD;
Thomas L. Hearn, MS; William O. Schalla, MS;
Harold W. Muir, MBA, Atlanta, Ga

268 **Human Immunodeficiency Virus Type 1 Proficiency Testing: The American Association of Blood Banks/College of American Pathologists Program**
Herbert F. Polesky, MD,
Margaret R. Hanson, MT(ASCP), SBB, Minneapolis, Minn

272 **Human Immunodeficiency Virus Type 1 Reference Materials**
Thomas A. O'Brien, PhD, Raritan, NJ

Continued on page 226

Continued from page 225

277 **Role of the Microbiology Laboratory in the Diagnosis of Opportunistic Infections in Persons Infected With Human Immunodeficiency Virus**
Calvin L. Strand, MD, New York, NY

284 **Biopsy Diagnosis in Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome**
Harry L. Ioachim, MD, New York, NY

295 **Transfusion of the Patient With Acquired Immunodeficiency Syndrome**
Klaus Mayer, MD, New York, NY

298 **The Significance of HIV-1—Indeterminate Western Blot Results in Blood Donor Populations**
Steven Kleinman, MD, Los Angeles, Calif

304 **Confidentiality and Informed Consent and the Acquired Immunodeficiency Syndrome Epidemic**
Susan L. Lentz, JD, Minneapolis, Minn

309 **Legal Liability for Transfusion Injury in the Acquired Immunodeficiency Syndrome Era**
Thomas F. Zuck, LLB, MD, Cincinnati, Ohio

316 **Physician Education: The Challenge of Acquired Immunodeficiency Syndrome**
Jared N. Schwartz, MD, PhD, Charlotte, NC

319 **The Epidemiology of Fear: Scientific, Social, and Political Responses to the Occupational Risk of Blood-Borne Infection**
Paul Bachner, MD, Port Chester, NY

324 **The Autopsy in Acquired Immunodeficiency Syndrome: How and Why**
Stephen A. Geller, MD, Los Angeles, Calif

330 **Current Risk of Transfusion-Associated Human Immunodeficiency Virus Infection**
Jay E. Menitove, MD, Milwaukee, Wis

335 **Current Safety of Clotting Factor Concentrates**
Jay S. Epstein, MD,
William A. Fricke, MD, Bethesda, Md

341 **Classified Advertising**

230 **Instructions for Authors**

CORRECTION

308 **A Review of the Histology, Ultrastructure, Immunohistology, and Molecular Biology of Extra-adrenal Paragangliomas**
Karl E. Kliewer, MD,
Alistair J. Cochran, MD, Los Angeles, Calif

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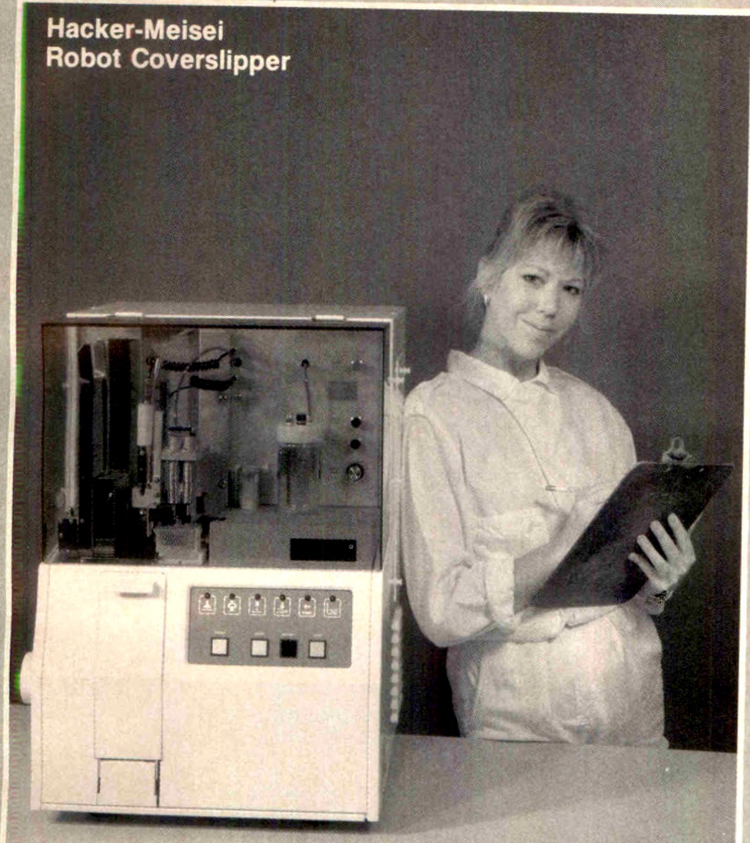

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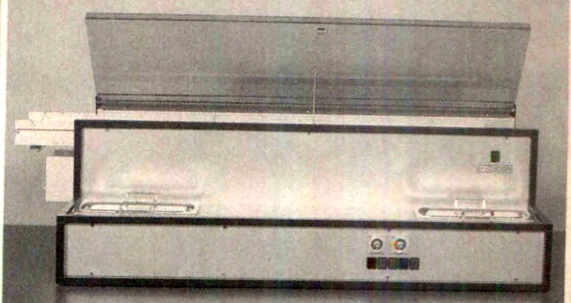

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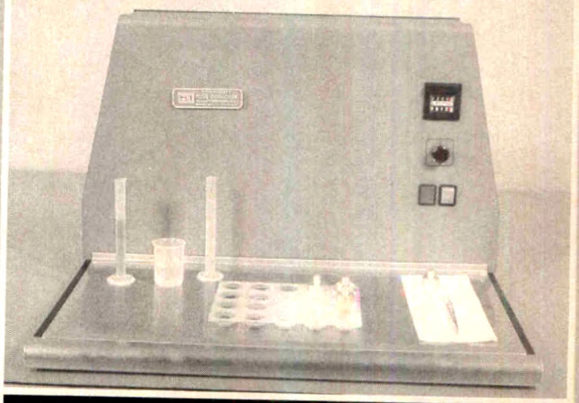

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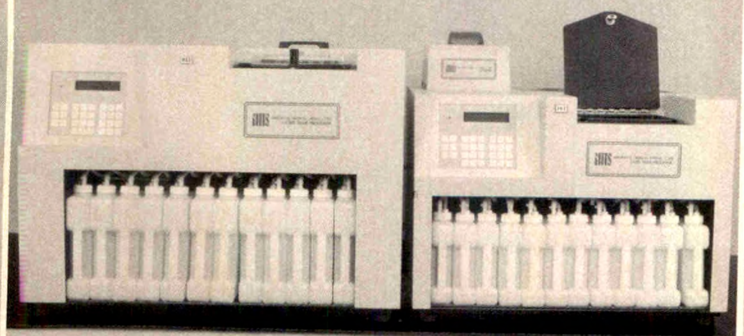

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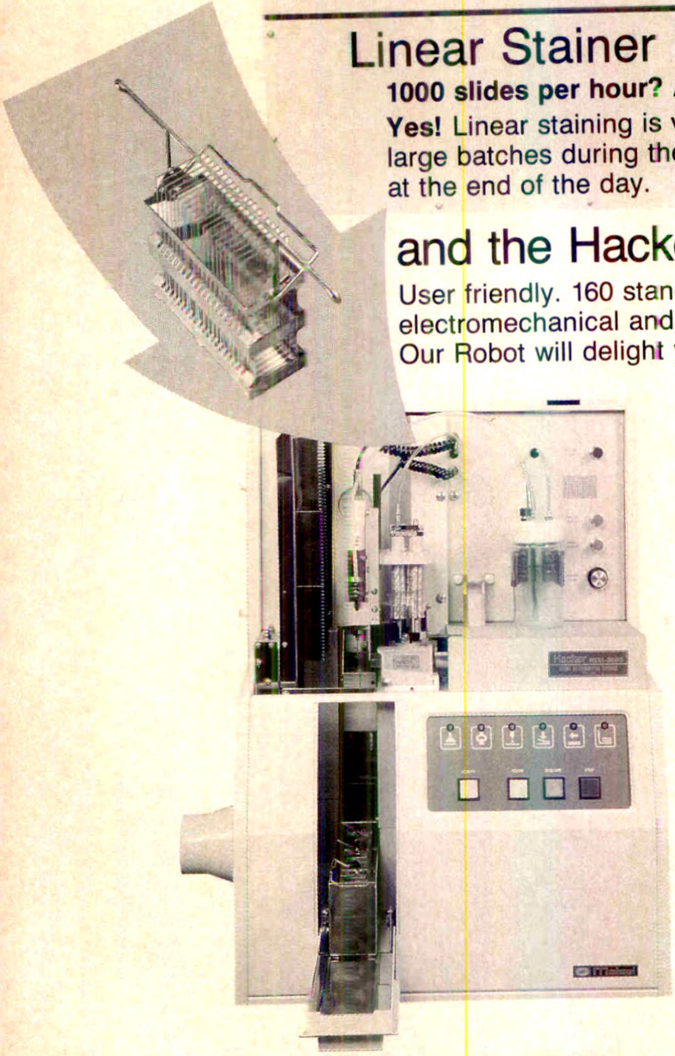

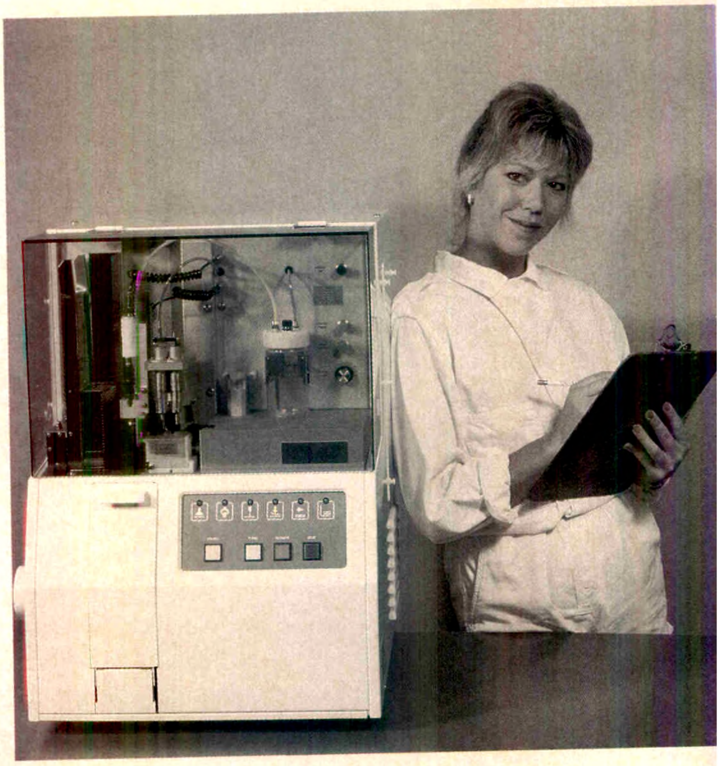

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Herbert F. Polesky, MD

The emergence of the human immunodeficiency virus (HIV) and the diseases associated with it have permanently modified medical practice. In the less than 10 years since it was first recognized in a small cluster of young gay males with *Pneumocystis carinii* pneumonia,[1] acquired immunodeficiency syndrome (AIDS) has been diagnosed in over 112 000 persons in the United States[2] and has occurred in over 100 countries. The exact number of HIV-infected asymptomatic individuals is unknown; however, it is estimated to be in excess of 1 million in the United States.[3]

The effect of this tragic epidemic on the practice of pathology has been most evident in the continuing debates about the safety of the autopsy, how to change laboratory practice to meet the requirements of "universal blood and body fluid precautions," and the best methods to ensure the safety of the "blood supply." The purpose of this conference was to bring together experts with diverse interests to review these issues and discuss current knowledge about AIDS and HIV infection with an emphasis on the areas of specific interest to the practice of pathology. In institutions caring for patients with AIDS and HIV infection, the impact of the disease is felt in every aspect of pathology practice. The articles in this issue of the ARCHIVES delineate the importance of laboratory services in the diagnosis, support, and monitoring of patients with AIDS. Included are discussions about the progress that has been made in testing, application of new technology to detect very early infection, and the problems of specificity, sensitivity, and interpretation of current test results.

In the short time since the discovery of HIV, a tremendous amount of information has been accumulated about the molecular biology of retroviruses. The many manifestations of infection with these viruses and the problems associated with the immunodeficient state of the infected host have provided unique challenges to the anatomic pathologist and the microbiologist. The AIDS epidemic has been particularly taxing to blood bankers, who since early 1983 took what they considered appropriate steps to eliminate potentially dangerous donors, yet are now faced with litigation from individuals transfused with blood from carefully selected, apparently healthy individuals who were subsequently identified as infected with HIV. Other legal issues and the potential for discrimination against persons with HIV infection have reemphasized the need to be aware of confidentiality of medical information and the importance of informed consent.

From the diversity of information presented at this conference and included in the following articles, several messages were recurrent: (1) AIDS will continue to be a major problem for the health care system and society. (2) The pathologist has a unique opportunity to provide leadership in educating the physicians and public he or she serves. (3) The pathologist should play a leading role in ensuring a reasoned response to this epidemic based on scientific knowledge rather than fear.

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The Pathobiology of HIV Infection

A Review

Roger D. Smith, MD

• Human immunodeficiency virus selectively infects lymphocytes and macrophages resulting in profound immunosuppression leading to opportunistic infections. This review deals with the virus/cell interaction and explains the apparent paradox of the global immune defect in the acquired immunodeficiency syndrome of a single subset of lymphocytes.

(*Arch Pathol Lab Med.* 1990;114: 235-239)

Since the identification of the human immunodeficiency virus (HIV) as the causal agent of the acquired immunodeficiency syndrome (AIDS), there has been an extraordinary amount of new information available regarding retroviruses as human pathogens. This review summarizes essential information concerning the virus and its interaction with cells, particularly as related to effects on the immune system. Cited references for the most part are other review articles that deal with the subject in greater depth. Also included is a brief summary of some unresolved issues that are the focus of intense interest and current research.

THE VIRUS

Human immunodeficiency virus preferentially affects cells of the immune system, eventually causing profound immunosuppression resulting in debilitating and often fatal opportunistic infections. As with other RNA retroviruses, HIV has the property of reverse transcription, resulting in a proviral DNA copy that integrates with host cell DNA. Such integration allows long periods of latency but ensures persistent, irreversible infection and, with activation, progressive and eventually fatal disease.[1,2] In order to understand the pathogenesis of HIV infection, it is necessary to consider the components of the infecting virus that are responsible for the virus life cycle and host response. Human immunodeficiency virus shares many features with other members of the lentivirus family of retroviruses, including visna virus of sheep, equine infectious anemia virus, and the feline immunodeficiency virus. All of these "slow virus" agents, including HIV in humans, cause a slowly progressive and inevitably fatal disease in their hosts. Human immunodeficiency virus is also related to recently identified primate retroviruses in the simian host that selectively infect T lymphocytes. Indeed, a second strain of HIV, designated human immunodeficiency virus type 2 (HIV-2), which is the predominant strain of the retrovirus infecting west Africans,[3] is more closely related to the simian virus than is the original HIV, now called human immunodeficiency virus type 1 (HIV-1), that predominates in the US population. Serological evidence and molecular homology of virus strains seem to indicate that the human virus originated from a simian virus transmitted in Africa.[3]

Virus particles can be readily identified by electron microscopy at the surfaces of cells productively infected with HIV (Fig 1). The virus has a dense cylindrical or bullet-shaped core encompassed by a lipid envelope that is partially derived from the surface cell membrane. The core contains two strands of genomic RNA (Fig 2) and various proteins given numerical designations based on their molecular weights. One core protein, p24, is of particular importance since it is unique to HIV and induces antibody that can be detected early in infection. The core also contains enzymes required for viral synthesis, including reverse transcriptase and an integrase, enabling transcription from viral RNA to DNA and integration of the proviral DNA into the host cell genome. The central core is surrounded

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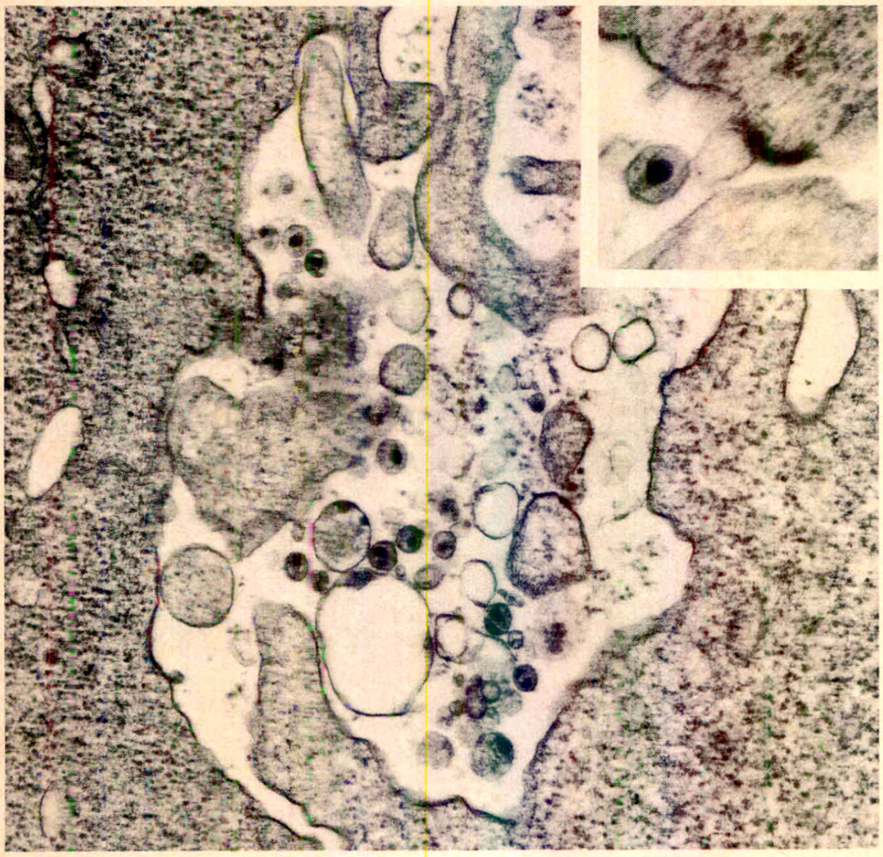

Fig 1.—Electron micrograph of lymphocyte infected in vitro with human immunodeficiency virus showing virus particles between two cells (uranyl acetate–lead citrate, original magnification ×15 000). Insert, virus particle and condensation of plasma membrane during virus budding (original magnification ×30 000).

by a lipid envelope that develops by budding from the surface of the infected cell (Figs 1 and 3). In addition to components of the host cell membrane, the envelope contains surface projections composed of two glycoproteins, gp120 and gp41. The HIV proviral genome has been well characterized (Fig 4). It is composed of three open-reading frames for the structural proteins comprising the core (*gag*), enzymes (*pol*), and the viral envelope (*env*). Regulatory sequences are clustered at each end of the structural genes as long terminal repeats. Long terminal repeat sequences contain targets for transcriptional factors and regulatory proteins that signal the initiation and progression of viral synthesis and are unique genetic components of retroviruses, serving as the junctional points for integration of the proviral genome into host DNA. Human immunodeficiency virus also has at least five additional genes, at least one of which down-regulates virus expression (*nef*) and two of which up-regulate HIV synthesis by counteracting *nef* repression. The *tat* gene plays a particularly significant role since it amplifies virus replication by encoding a protein that is a potent transactivator of HIV gene expression. The regulatory genes of the provirus are of particular interest and importance because their expression regulates changes from a latent to a productive infection and, ultimately, the development of AIDS.[4]

VIRUS CELL INTERACTION

Even before the identification of HIV as the causal agent of AIDS, it was noted that, with the progression of the disease, there was a selective depletion of helper/inducer T lymphocytes that express the CD4 phenotypic marker, eventually resulting in profound immunosuppression. Subsequently, it was found that the CD4 protein serves as a high-affinity receptor for the gp120 of the viral envelope, accounting for selective infections of T4 lymphocytes. Although the T4 cell seems to have the highest density of CD4 receptors, portions of the CD4 molecule have been identified on other cells, such as monocytes and macrophages, that also play a central role in the pathogenesis of HIV infection. After the virus binds to the CD4 molecule, the viral core is internalized (Fig 3). In addition to gp120, the transmembrane glycoprotein, gp41, of the virus envelope is required for viral entry. There are likely to be other cell surface proteins involved, since mouse cells transfected with the CD4 gene expressing the human CD4 protein effectively bind HIV but do not internalize the virus. After internalization, viral genomic RNA is transcribed to proviral DNA, which then either accumulates in the cytoplasm of the cell in an unintegrated circular form or becomes integrated into the host chromosome DNA. One unusual feature of HIV infection, as compared with other retroviruses, is the accumulation of unintegrated viral DNA in the infected cell. Since most other retroviruses are not cytopathic, it has been suggested that the accumulation of proviral DNA is an important factor in the cytopathogenicity of HIV infection, although the specific mechanism responsible for cell lysis is not yet known.

After integration of the proviral DNA, there may be a latent phase with restriction of the viral cycle until the infected cell is activated. Various agents have proved to be effective activators in vitro, including mitogens, other antigens, and heterologous viral genes.[1,4] A common mechanism resulting in activation involves transactivation with the production of cellular DNA-binding proteins that interact with the long terminal repeat sequences of the proviral HIV. Once cell activation occurs, probably accompanied by demethylation of proviral DNA, there is transcription of viral genomic RNA and messenger RNA, followed by protein synthesis, processing, and virus assembly and release with budding of mature virions from the cell surface.

Although the long and variable period

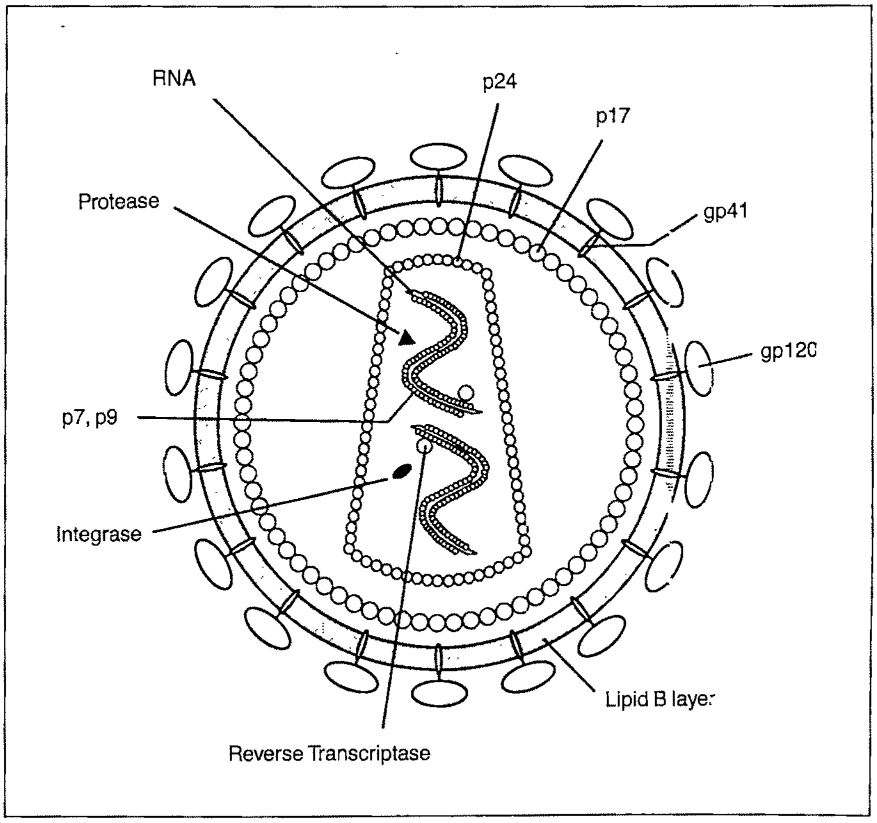

Fig 2.—Virion structure of human immunodeficiency virus (HIV) as diagrammed in cross-section. The envelope of the virion is composed of a lipid bilayer with glycoproteins, gp120 and gp41. The core contains two strands of RNA, enzymes, and core proteins, one of which (p24) is unique to HIV and induces an antibody response that can be detected early in infection.

of clinical latency, with a mean of approximately 8 years, is undoubtedly related to prolonged and silent integration of the HIV proviral DNA, there is a chronic or low-level production of HIV in infected individuals. It is not known whether this is due to a release of HIV from a few activated T cells undergoing lysis or low-level release of infectious virus from lymphocytes and particularly monocyte/macrophages that contain stably integrated proviral DNA. However, there is an accumulated body of evidence to show that activation of T4 lymphocytes with virus release and lysis of the host cell are important factors in signaling the progression of the disease.[4]

CRITICAL ROLE OF THE T4 LYMPHOCYTE

Epidemiologic and clinical studies have indicated that a decrease in the absolute number of T4 lymphocytes signals the emergence of opportunistic infections and correlates with the progression of the disease.[5] Although depletion of T4 lymphocytes with T4:T8 ratio inversion is almost always noted with AIDS, a qualitative or functional defect of selective subsets of T4 cells in asymptomatic seropositive HIV-

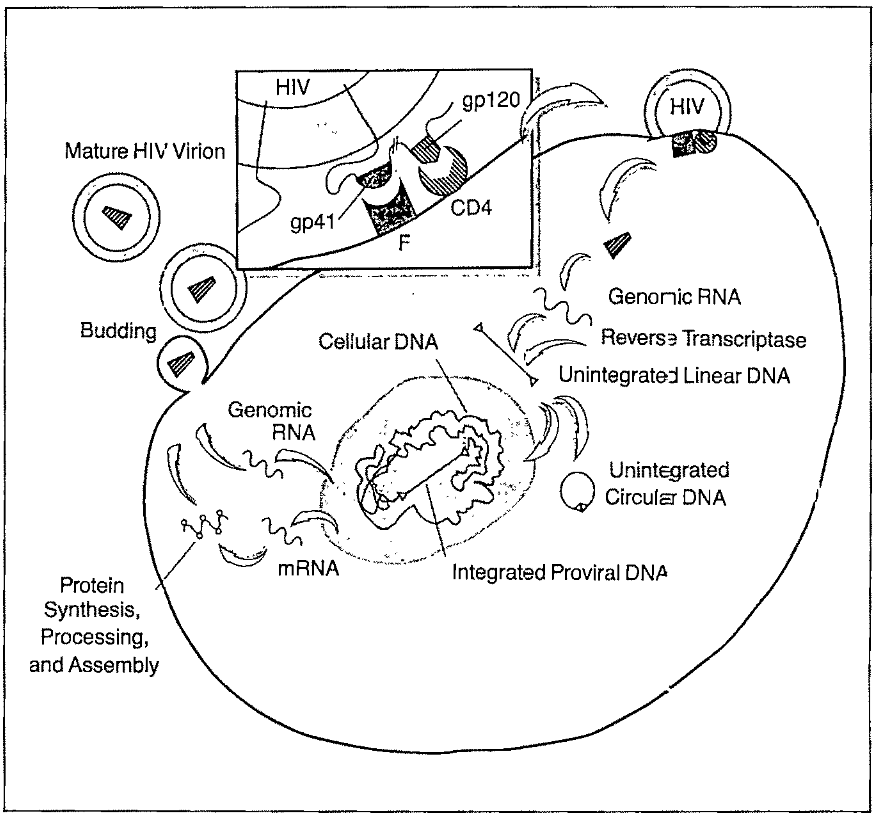

Fig 3.—Diagram of the life cycle of human immunodeficiency virus (HIV) in the T4 lymphocyte. The gp120 of the virus binds to the CD4 receptor on the surface of the lymphocyte and the core of the virion is internalized, requiring the transmembrane viral glycoproteins of gp41 and another putative cell surface component (F). The internalized genomic viral RNA is transcribed to proviral DNA that either accumulates in the cytoplasm or becomes integrated into the host cell DNA. With activation, there is transcription of viral genomic and messenger RNA (mRNA) leading to protein synthesis, virus assembly, and release from the cell surface by budding.

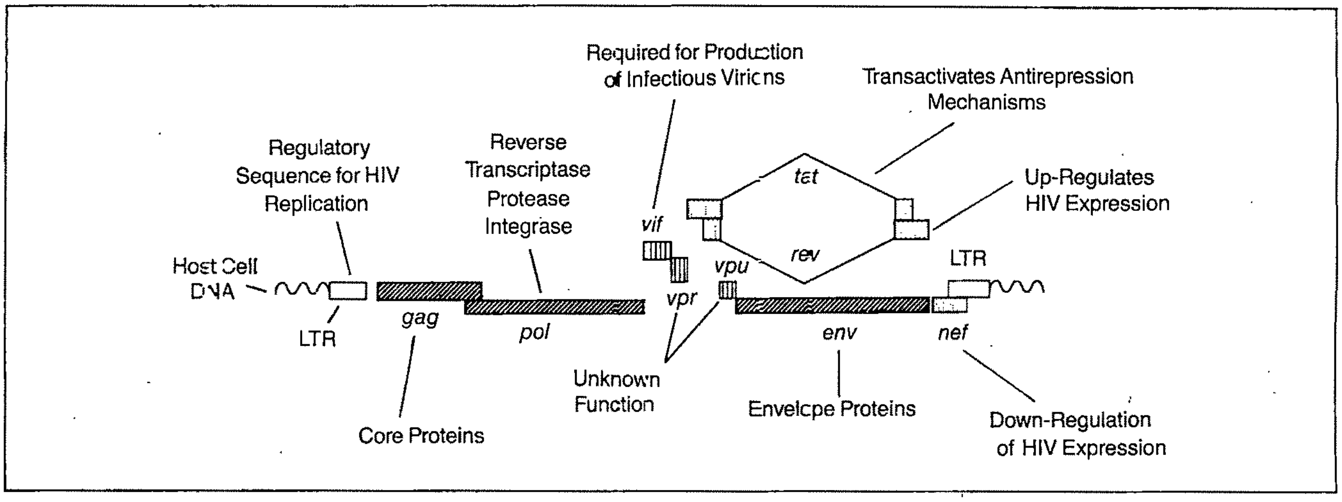
Required for Production of Infectious Virions
Transactivates Antirepression Mechanisms
Regulatory Sequence for HIV Replication
Reverse Transcriptase Protease Integrase
tat
Up-Regulates HIV Expression
vif
Host Cell DNA
vpu
rev
LTR
vpr
LTR
gag
pol
env
nef
Unknown Function
Envelope Proteins
Core Proteins
Down-Regulation of HIV Expression

Fig 4.—Diagram of the proviral DNA genome of human immunodeficiency virus (HIV). The dark bars are structural genes for the viral core (*gag*), polymerases (*pol*), and envelope (*env*). The other genes are for regulation of transcription. The long terminal repeat (LTR) sequences contain targets for transcriptional control and regulatory proteins and serve as junction points for integration into host DNA.

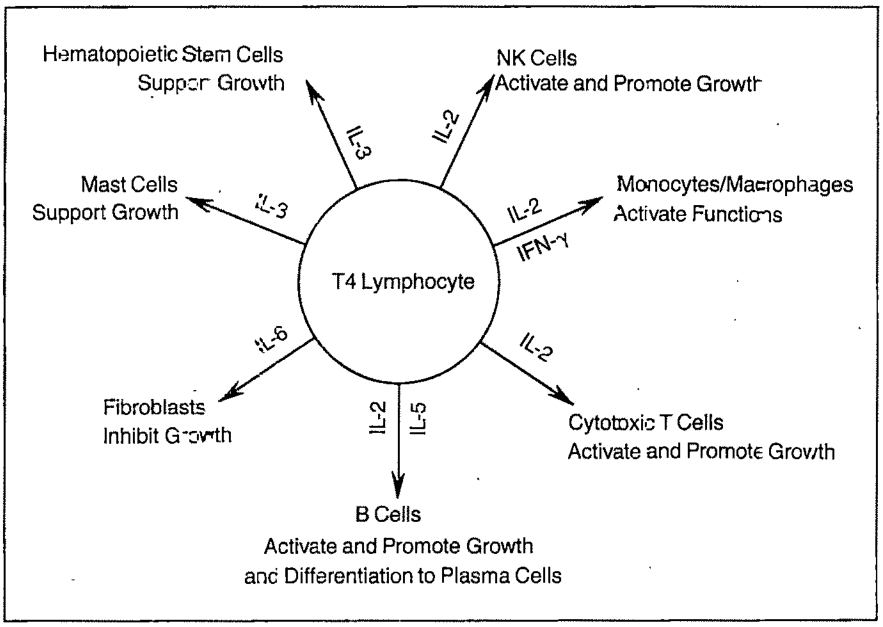

Fig 5.—Diagram illustrating the central role of the T4 lymphocyte in the immune response. Arrows represent lymphokine inducers (interleukins 2, 3, 5, and 6 [IL-2, -3, -5, and -6]) that are polypeptides released by activated T4 cells that have specific effects on target cells. Interferon gamma (IFN-γ) from T4 cells activates macrophages. NK indicates natural killer cells.

infected individuals has also been demonstrated.[6] Whether this is due to lysis and depletion of selective subpopulations of T4 cells or noncytopathic infection and inhibition of cell function by subunits of HIV, particularly the envelope protein or some other inhibitory mechanism, is not yet resolved. The T4 lymphocyte is the central critical cell involved directly or indirectly in the induction of many immunologic functions (Fig 5), particularly by release of polypeptide lymphokines.[7] As an example, interleukin 2 released from T4 lymphocytes is needed for clonal expansion of antibody-producing B lymphocytes in response to a soluble antigen. Although patients with AIDS frequently have hypergammaglobulinemia, probably because of stimulation of B lymphocytes by Epstein-Barr virus or cytomegalovirus, without adequate interleukin 2 from T4 lymphocytes, these patients are poor antibody producers. Interleukin 2 is also needed for clonal expansion of cytotoxic lymphocytes in response to antigens. The opportunistic bacterial, fungal, parasitic, and viral infections that occur in AIDS are principally the result of defective antibody and cytotoxic lymphocyte responses because of HIV infection of T4 lymphocytes. The defect in number and function of T4 cells resulting in decrease of inductive signals to various limbs of the immune response system account for the apparent paradox of a global immune defect as observed in AIDS, despite selective infection of a single subset of lymphocytes.

ROLE OF THE MONOCYTE/MACROPHAGE

In addition to functional defects in macrophages that may be secondary to a deficiency in T4-lymphocyte inducers, monocytes/macrophages also play a primary role in the pathogenesis of HIV infection. Although there are subsets of macrophages that have CD4 receptors, CD4-negative monocytes can also become infected, probably by receptor-mediated endocytosis possibly involving Fc receptors with facilitation of virus absorption by IgG antibody to HIV.[8] However, unlike T4 lymphocytes, macrophages are relatively resistant to the cytopathic effects of the virus but can carry incorporated proviral genome, synthesize and release infectious HIV, and serve as a reservoir and transport vehicle for the spread of infection to various organs. Macrophages appear to be the principal HIV-infected cell in the brain, and the release of monokines from infected macrophages has been implicated as a putative neurotoxic agent responsible for some of the neuropsychiatric manifestations associated with HIV infection.[9] Further, secretion of interleukin 1 and tumor necrosis factor by infected macrophages is likely to be responsible for the fever and cachexia that occur in many patients with AIDS.

SOME UNRESOLVED ISSUES

One of the most puzzling and disturbing biological properties of HIV is its genetic instability resulting in the emergence of new strains. Even individual isolates of HIV-1 from a given patient are composed of populations of genetically and biologically distinct variants.[10] Because the change of a single amino acid residue in the gp120 of the virus envelope may result in loss of neutralizing activity of an antibody, genetic variation is such a major obstacle to immunoprophylaxis that vaccine development may not be a practical approach to prevention. This genetic shift may be responsible for the apparent paradox of disease progression in patients, despite high levels of antibody that may be neutralizing in vitro. Indeed, evaluation of repeated HIV isolates from patients during the course of their infection suggests that progression may be the result of the emergence of new genetic variants of HIV that are more cytotoxic for target cells.[11] Genetic variation and selection are also probably responsible for the reduced sensitivity to zidovudine (azidothymidine) recently reported to occur during prolonged therapy.[12]

There is an increasing body of evidence to indicate that the virus load may be the most important determinant of clinical disease.[13] Although the laboratory models of latency and activation of HIV synthesis in vitro have led to hypotheses explaining disease progression, there is a need for analysis of patterns of viral gene activation and expression in vivo to answer some of the critical questions. Unfortunately, a good applicable animal model is yet to be identified. Although genetic activation with increase in HIV synthesis proceeding to lysis of the T4 lymphocyte is undoubtedly a critical event in disease progression and particularly immunosuppression, the early manifestations of disease involve a variety of mechanisms other than lysis of T4 lymphocytes. These include cytotoxic immune responses, autoimmune reactions in the host, the effects of viral proteins on uninfected cells, and the inhibitory or enhancing effect on synthesis and release of cytokines and leukotrienes. Whether these or other mechanisms are responsible for the development of isolated organ system disease, such as cardiomyopathy, nephropathy, or dementia, in HIV-infected but otherwise asymptomatic individuals is still unknown.

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The Western Immunoblot Procedure for HIV Antibodies and Its Interpretation

Roger Y. Dodd, PhD, Chyang T. Fang, PhD

• The Western blot is frequently used to confirm human immunodeficiency virus type 1 (HIV-1) screening tests as it permits the identification of antibodies to individual component polypeptides of HIV-1. The procedure is described and different criteria for evaluation of HIV-1 Western blots are compared. The significance and management of indeterminate blot patterns are discussed.

***(Arch Pathol Lab Med.* 1990;114: 240-245)**

Although the specificity of most enzyme-linked immunosorbent assays (ELISA) for antibodies to the human immunodeficiency virus (HIV) exceeds 99.8%,[1] they are often used to test populations with a very low prevalence of HIV infection. In these circumstances, the positive predictive value of the test may be 10% or even less. Consequently, it is critically important to employ supplementary test procedures to validate the HIV infection status of individuals with reactive screening results. At the same time, it must be realized that false-positive screening test results will generally occur with the same frequency in any population tested. Therefore, even in populations with a high prevalence of HIV infection, some screening test results will show false-positive results. Thus, as a general principle, any reactive screening result for HIV antibody must be confirmed prior to diagnosis or notification of the tested subject.

There are a variety of methods for confirmation of HIV screening assays. Currently, however, the Western blot test and indirect immunofluorescence are most frequently used for this purpose. The Western blot is a technique that permits the identification of antibodies directed to individual structural polypeptides of HIV. The peptides are electrophoretically separated and transferred ("blotted") to nitrocellulose where they are stabilized. The nitrocellulose is exposed to positive serum samples, and reactive antibodies can be visualized and located by use of appropriately labeled antiglobulins. Properly used and interpreted, Western blot analysis can correctly identify the vast majority of HIV-infected individuals. However, a proportion of Western blot assays will generate ambiguous results (indeterminate patterns) that require additional resolution before the presence or absence of HIV infection can be assured. The proportion of readable blots with indeterminate patterns found among ELISA-positive samples may vary from almost none in high-prevalence populations to 50% or more among blood donors[2] (C.T.F., unpublished observations, October 1989).

In this article, we discuss the theory and technology of the Western blot analysis, current criteria for evaluation of blots, and potential approaches to the resolution of indeterminate blots.

STRUCTURAL PROTEINS OF HIV

In common with other retroviruses, HIV has three major structural genes, *gag*, *pol*, and *env*, and a variety of regulatory genes. Conventional Western blot tests for HIV are designed only to detect antibodies to the structural gene products, although some specialized blots have been designed to detect antibodies to one or more regulatory gene products, such as *nef*.[3] The *gag* or group antigen gene codes for those polypeptides comprise the core, or nucleocapsid of the virus. In simple terms, the primary product of the *gag* gene is a 55-kd polypeptide that is processed, during viral maturation, to smaller polypeptides, including those of 24 and 17 kd. The p55, p24, and p17 polypeptides should be present in viral lysates and should be represented on the nitrocellulose Western blot strip.

The *pol*, or polymerase gene codes for the complex of enzymes, is responsible for the process of reverse transcription, ie, the translation of the viral RNA genome to a DNA sequence within the infected cell.[4] Key polymerase polypeptides that should be expressed on a blot are the p66 reverse transcriptase enzyme and its processed derivative, p51. In addition, the *pol* gene specifies a 31-kd polypeptide with endonuclease activity, which must also be present on a blot.

The *env*, or envelope gene, specifies the structure of the polypeptide component of the membrane glycoproteins of the retrovirus. The envelope peptides are glycosylated by cellular processes and are then found in the viruses as glycoproteins. The *env* gene specifies a 160-kd parent glycoprotein that comprises a 120-kd surface glycoprotein and a 41-kd transmembrane component. Although the *env* glycoproteins appear to be subject to considerable variability in their primary sequences, there are some highly conserved areas of considerable importance, which will be discussed elsewhere. A Western blot strip should include all of the *env* products, gp160, gp120, and gp41. Interestingly,

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it has recently been suggested that in some blots those components with electrophoretic mobility equivalent to 120 and 160 kd may actually represent a trimer and a tetramer of gp41, respectively.[5]

As a result of the mechanisms inherent in glycosylation, individual glycoprotein molecules, although identical in peptide structure, will not be homogeneous with respect to the number of saccharide residues. Consequently, on electrophoresis, glycopeptides tend to emerge as somewhat diffuse bands, and this diffused character is an extremely important component of the proper recognition and interpretation of *env* bands on a blot.

In the case of human immunodeficiency virus type 2, the corresponding polypeptides have slightly different molecular weights. The *gag* gene products are p55, p26, p16, and p12. The *pol* gene codes for a p68 reverse transcriptase and a p34 endonuclease. The *env* glycoprotein precursor is specified as gp 120/130, comprising an external gp105/120 glycoprotein and a transmembrane gp 36/41.[6]

WESTERN BLOT TECHNOLOGY

The initial use of the Western blot procedure for HIV antibodies was described by Sarngadharan and coworkers in 1984.[7] In general, most HIV blot protocols are derived from this procedure.[8] Although a number of laboratories still prepare Western blot tests for their own use, prepared kits are readily available from a number of manufacturers. At the time of writing, one product, manufactured by Du Pont and Biotech, Rockville, Md, was licensed by the US Food and Drug Administration.

The first stage in the preparation of a Western blot test is growth and purification of HIV. The purified virus is lysed by an appropriate detergent and is reduced and treated with sodium dodecyl sulfate. This process releases the individual peptides from the virus and maximally extends each peptide so that its molecular size is directly proportional to its molecular weight. The treated viral lysate is then applied to a polyacrylamide gel and electrophoresed in the presence of sodium dodecyl sulfate. The electrophoretic mobility of each peptide is inversely proportional to its molecular weight. Thus, the lower-molecular-weight peptides travel further. Once the polyacrylamide gel electrophoresis is complete, the peptides are banded in positions that are related to their molecular weight. These bands are transferred to a sheet of nitrocellulose paper to stabilize them and to permit further manipulations. This process is achieved by placing the nitrocellulose sheet on the face of the acrylamide gel and applying an electrical charge through the thickness of the gel. The peptides migrate through and out of the gel and are trapped on the nitrocellulose in a pattern that exactly replicates what is originally on the gel. The blotted nitrocellulose paper is washed and dried and cut into strips in such a fashion that the strips bear the overall band pattern. In commercially available Western blot kits, the strips have already been prepared.

In order to perform the test, a dilution of the serum sample is incubated with the strip. If antibodies to determinants on the peptides are present, they will adhere to the peptide bands on the strip. After incubation, the strips are washed and then exposed to an antiglobulin conjugate. Most frequently, the conjugate is horseradish peroxidase, although it is also possible to use radioactively labeled antiglobulin. The conjugate detects the adherent antibodies and its presence is visualized by a reaction that generates an insoluble, colored precipitate in the case of the peroxidase conjugate. The substrates that are generally used are 4-chloro-1-naphthol or diaminobenzidine. Radiolabeled antiglobulin is detected by autoradiography.

Once the reaction is complete, the Western blot strip can be washed, dried, and evaluated. In performing Western blot determinations, it is important to include appropriate control samples. In addition to positive and negative control samples, it is advisable to include a weak-positive sample, which can serve (as is the case with the Du Pont blot) as an intensity reference against which sample bands may be evaluated. In the case of blots that are being prepared in-house, it is important to include molecular-weight standards, at least until experience is gained. In addition, the acrylamide gel should be stained after electrotransfer in order to assure that the peptides have been completely transferred to the nitrocellulose.

Blot patterns for investigational samples must be read with careful reference to established control samples. As a minimum, the presence, or absence, of bands in the *gag*, *pol*, and *env* complex must be read, ie, p17, p24, and p55; p31, p51, and p66; and gp41, gp120, and gp160. Bands may also be apparent in other positions. Although they may not have any diagnostic significance, it is generally accepted that only blots without any visible bands can be scored as negative. The presence of any bands requires that a blot be read either as positive or indeterminate, according to the criteria used.

It is known that blots may include non–viral peptides. Reactions to such non–viral peptides can confuse the reading of a blot, and it is important to avoid confusion with viral bands. Most particularly, actin, a component of the cells used to grow the virus, may be present and can result in a *sharp* band in the same area as gp41. A gp41 band must be characteristically diffuse to be regarded as legitimate, although it should be noted that a sharp band in the p41 region may relate to infection with human immunodeficiency virus type 2. Another potentially confusing reaction may occur around the 51- to 55-kd region; this is thought to represent an antigen of the HLA complex. Clearly, there is potential for confusion with the p51 and p55 viral bands.

INTERPRETIVE CRITERIA

As noted above, a blot that has no visible bands is interpreted as *negative* for HIV antibodies, and the subject or patient may be considered free of HIV infection. The key to reading a blot with visible bands is the criterion that is used to define a positive result. Over the years, there have been a number of different sets of such criteria. There has been considerable discussion and controversy about interpretive criteria, and, even now, there does not seem to be a uniform consensus as to the ultimate standard.

When the Western blot was first described as a supplementary test for HIV

Table 1.—Interpretive Criteria for Western Blot Tests

Organization*	Minimum Band Requirements for a Blot to Be Termed "Positive"
ASTPHLD/CDC[14]	Two of p24, gp41, or gp120/160
CRSS[15]	p24 *or* p31 *and* one of gp41 or gp120/160
Du Pont/Biotech[8]	p24 *and* p31 *and* one of gp41 or gp120/160
ARC[8]	one of p17, p24 or p55 *and* one of p31, p51 or p66 *and* one of gp41 or gp120/160

*ASTPHLD indicates Association of State and Territorial Public Health Laboratory Directors, Iowa City, Iowa; CDC, Centers for Disease Control, Atlanta, Ga; CRSS, Consortium for Retrovirus Serology Standardization, Davis, Calif; and ARC, American Red Cross, Washington, DC.

ELISA, the US Public Health Service published a document that included its recommendation that a blot which included a band at the p24 and/or gp41 region should be read as positive.[9] Experience has shown that this criterion is inappropriate, inasmuch as single bands at p24 are frequently observed in uninfected individuals.[10-12] Subsequently, a recommendation from the National Institutes of Health, Bethesda, Md, suggested that bands at both p24 and gp41 were required to define a blot as positive. This criterion is highly specific, but does lack sensitivity.

These early criteria were developed prior to a time when blots routinely included glycoproteins of high molecular weight. Once reactions to these glycoproteins could be reliably detected, a variety of newer criteria have been proposed. These criteria are outlined in Table 1. In essence, they fall into two groups: The first group of criteria are essentially pragmatic, placing strong reliance on the presence of bands to glycoproteins. These criteria are those proposed by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), Iowa City, Iowa,[3] and subsequently endorsed by the Centers for Disease Control (CDC), Atlanta, Ga,[14] and those proposed by the Consortium for Retrovirus Serology Standardization (CRSS), Davis, Calif.[15] The major difference between these criteria is that CRSS does require the presence of bands to the products of two genes, whereas the ASTPHLD/CDC criterion could define a sample as positive on the basis of the presence of antibodies only to *env* gene products. Interestingly, if the apparent gp120 and gp160 bands actually represent multimers of gp41, a sample could be defined as positive on the basis of antibodies to gp41 alone.[14] The second group of criteria require the presence of antibodies to the products of each of the three structural genes. Of course, these criteria are also developed on the basis of careful evaluation and analysis of actual patterns, but have the theoretical advantage that antibodies to multiple independently expressed gene products must be present, implying the potential for increased specificity. The criteria for the evaluation of the Du Pont Western blot test have been approved by the Food and Drug Administration. They call for the presence of antibody bands to p24, p31, and one of the *env* glycoproteins, although it is recognized that more relaxed criteria may be justified in the context of additional information about the patient[8] (product insert, Du Pont Western blot kit for antibodies to HIV). The American Red Cross, Washington, DC, has adopted a criterion that calls for the presence of antibodies to at least one product from each of the three structural genes.[8]

Although these criteria appear to be very dissimilar, the nature of the immune response to HIV is such that, in most cases, a blot that is positive by one of the criteria will also be positive by the others. This is particularly true for individuals in the asymptomatic phase of infection. However, in the earliest phases of infection, antibodies to different viral components do develop sequentially, and there is a reduction in the levels of antibodies to some of the viral components during the symptomatic stages of the disease.[8] At these early and late phases, the use of different blot criteria may be more likely to generate different interpretations. In particular, the response to p31 is not consistent, although we have observed that antibodies to p66 are almost always present if those to p31 are absent.

Indeterminate blot patterns cause the most difficulties in interpretation. Although there are some patterns that are thought to be unrelated to HIV infection, any indeterminate blot requires additional serologic, virologic, or clinical information for proper interpretation. An individual without symptoms related to the acquired immunodeficiency syndrome (AIDS) who has an indeterminate pattern that remains unchanged in samples drawn 3 to 6 months apart is amost certainly not infected with human immunodeficiency virus type 1 (HIV-1).[14] This area will be discussed in greater detail below.

BAND PATTERNS OBSERVED IN WESTERN BLOTS

Table 2 shows the results of a study evaluating the distribution of bands observed when performing blots on samples taken from patients with AIDS and from blood donors.[8] In this table, the Red Cross criterion was used to define a positive specimen. Particularly noteworthy is the uniform presence of *env* bands of high molecular weight in all positive samples and their absence among samples showing indeterminate results. In addition, although the p31 band seems to be highly specific, it is absent in 5% of positive donors or 6% of patients with AIDS. Table 3 illustrates the pattern of development of antibody reactivities during seroconversion in one typical sequence of samples. In general, antibodies to *gag*-encoded proteins appear first, followed closely by those to *env* glycoproteins. In this series, using the Du Pont blot, the first *pol* reactivity to appear is to p66.

A number of groups have reviewed the impact of different reading criteria for the Western blot (Rapoza et al, unpublished observations).[14,15] These studies have most frequently been used to support a particular interpretive standard. It is, perhaps more important to emphasize that there is a group of criteria that generate remarkably similar outcomes when they are compared on the same sample population. Table 4 illustrates this point, using data from the literature and from our own evaluation of data from 951 selected blood donor samples. It is clear that the differences between the ASTPHLD/CDC, the CRSS, and the American Red Cross cri-

Table 2.—Frequency of Band Expression in Western Blot Tests*

	Samples With Detectable Bands, %		
Donors Band	Patients With AIDS (n = 127)	Seropositive Donors† (n = 914)	Indeterminate‡ (n = 969)
gag			
p17	84	89	38.7
p24	98	99	18.2
p55	99	99	79.4
pol			
p31	94	95	0.0
p51	99	98	1.8
p66	99	99	1.8
env			
gp41	100	100	0
gp120	100	99	0
gp160	100	100	0
All bands	76	85	0

*From Sandler et al[8]. AIDS indicates acquired immunodeficiency syndrome.

†Blood donors with reactivity to at least one band from each of the *gag*, *pol*, and *env* genes.

‡Blood donors with visible bands that do not meet the criteria noted above.

Table 3.—Development of Western Blot Pattern During Seroconversion in a Single Individual

	Western Blot Pattern†								
	gag			*pol*			*env*		
Day Sampled	p17	p24	p55	p31	p51	p66	gp41	gp120	gp160
0	−†	±	−	−	−	−	−	−	±
7	−	+	−	−	−	−	−	−	±
9	−	+	−	−	−	±	−	−	+
16	−	+	−	−	±	±	−	±	+
21	−	+	−	−	±	+	−	+	+

*First available sample.

†Minus sign indicates no visible band; plus sign over minus sign, band with an intensity less than that of the weak control sample; plus sign, band with intensity equal to or greater than that of the weak control.

Table 4.—Outcome of Using Different Criteria for the Evaluation of Western Blot Tests*

		% Classified as Positive by the Criteria of			
Study Population	No. With Detectable Bands	ASTPHLD/CDC	CRSS	Du Pont/Biotech	ARC
CDC[14]	184	90.8	85.9	73.4	82.1
CRSS[15]	646	86.7	83.1	70.6	86.8
ARC[8]	951	50.5	49.7	46.8	49.5
Total	1781	67.8	65.5	58.2	66.4

*ASTPHLD indicates Association of State and Territorial Public Health Laboratory Directors, Iowa City, Iowa; CDC, Centers for Disease Control, Atlanta, Ga; CRSS, Consortium for Retrovirus Serology Standardization, Davis, Calif; and ARC, American Red Cross, Washington, DC.

teria are relatively small, but the criteria included in the product insert of the Du Pont blot kit appear to be less sensitive. However, it is important to note that those criteria are recommended for use in circumstances where specificity is paramount (ie, notification of blood donors) and where there is no other supporting or diagnostic information. However, it seems likely that a somewhat-less-rigorous three gene criterion, such as that of the American Red Cross, may be as specific and, perhaps, more sensitive. Finally, skill and experience are required to read blots effectively and accurately. It may not be possible to formalize every aspect of the judgment used to read a difficult blot, other than to reemphasize the need for detailed assessment of the placement and nature of each observed band. One unresolved issue is the need for an intensity standard. While this is an integral component of the Du Pont procedure and is certainly extremely important in the standardization of reading, many laboratories do not rely on any intensity criteria. Certainly, there are cases where strict adherence to requirements for intensity may result in the improper classification of a positive sample as an indeterminate sample.

A Western blot test cannot be readily interpreted when bands are present but the criteria for positivity are not met. Such patterns are defined as indeterminate. In testing samples that are repeatedly reactive in ELISA, the frequency of indeterminate blot patterns may be as high as 20% in populations with a low prevalence of HIV infection.[16] Interestingly, even among persons whose tests show negative results for HIV antibodies in ELISA, up to 15% of persons may have tests showing bands on a blot, even though they are clearly not infected with HIV (product insert, Du Pont Western blot kit for antibodies to HIV). The causes for these bands are not clear, but potential explanations are discussed elsewhere in this issue of the ARCHIVES.[17] Table 5 shows the band patterns found in reviewing 480 blots from ELISA-positive blood donors defined as indeterminate on the basis of the Red Cross criterion for a positive blot reading.

RESOLUTION OF INDETERMINATE BLOTS

Although it has been recommended that laboratories should report test results as positive, indeterminate, or negative,[14] an indeterminate outcome cannot be regarded as complete and every attempt should be made to resolve the laboratory finding. When appropriate additional viral or clinical information is available, certain incomplete blot patterns (particularly those characterized by weak or absent *gag* reactivities) may indeed be supportive of an overall diagnosis of HIV infection. However, in our opinion, an indeterminate blot should not be interpreted as evidence of HIV infection solely on the grounds that the subject has an AIDS risk factor.

In the case of an asymptomatic indi-

Table 5.—Distribution of Western Blot Reactivity Among 480 Blood Donors With Patterns Classified as Indeterminate

Band Pattern										
gag			*pol*			*env*				
p17	p24	p55	p31	p51	p66	gp41	gp120	gp160	No.	%
+	...	...	.	...	...	...	...	...	49	10.2
..	+	...	.	...	...	...	...	...	150	31.3
..	...	+	.	...	...	...	...	...	113	23.5
+	+	...	.	...	...	...	...	...	4	0.8
+	...	+	.	...	...	...	...	...	19	4.0
..	+	+	.	...	...	...	...	...	23	4.8
..	...	...	–	...	...	...	...	...	3	0.6
..	...	...	.	+	...	...	...	...	3	0.6
..	...	...	.	...	+	...	...	...	7	1.5
..	...	...	.	+	+	...	...	...	4	0.8
..	...	...	.	...	...	...	...	+	5	1.0
+	...	...	–	...	...	...	...	...	2	0.4
+	...	...	.	+	...	...	...	...	1	0.2
+	...	...	–	+	...	...	...	...	2	0.4
+	...	...	..	+	+	...	...	...	1	0.2
..	+	...	.	+	...	...	...	...	1	0.2
..	+	...	.	+	+	...	...	...	3	0.6
...	...	+	.	+	...	...	...	...	41	8.5
...	...	+	–	+	...	...	...	...	1	0.2
+	...	+	–	...	...	...	...	...	16	3.3
..	+	+	.	+	...	...	...	...	3	0.6
..	+	+	.	...	+	...	...	...	1	0.2
+	...	...	..	...	...	...	+	+	1	0.2
..	+	...	.	...	...	...	...	+	2	0.4
+	+	...	.	...	...	...	+	+	2	0.4
+	+	+	.	...	...	...	+	+	3	0.6
..	...	...	.	...	+	...	+	+	1	0.2
Unreadable			..	...	...	...	...	...	19	4.0
Total									480	

vidual, the major difficulty in interpreting indeterminate blot patterns (particularly those characterized by the presence of reactivity to the *gag* peptide p24) is the possibility that the pattern signifies early development of antibodies during seroconversion. In fact, this outcome appears to be infrequent in low-risk populations—in our study, only about 1% of voluntary donors with indeterminate patterns are found to be in the process of seroconversion. Kleinman et al[2] reported that 5 of 93 donors with an indeterminate blot pattern were actually infected with HIV. On the other hand, in prospectively followed, high-risk populations, a high proportion of indeterminate blot patterns may reflect seroconversion. The simplest way to determine whether an indeterminate pattern is associated with seroconversion is to evaluate a second sample, drawn after a suitable time period. Seroconversion will be complete and a positive Western blot pattern will have evolved. Most authorities recommend that the second sample should be drawn 3 to 6 months after the first; however, the actual process of seroconversion is rapid and a lesser waiting period should be adequate, as discussed by Kleinman elsewhere in this issue of the ARCHIVES.[17]

Conversely, many studies have shown that a stable indeterminate blot pattern is almost certainly not due to infection with HIV-1.[10-12] Thus, individuals who, on follow-up, have retained the indeterminate pattern may be advised to this effect.[14] However, the requirement for a second sample is undesirable, since it delays the final result and will, in most circumstances, result in justifiable concern for the individual who is being tested. Thus, it would be beneficial to resolve an indeterminate blot pattern by additional testing on the initial sample. We have investigated this possibility by reviewing serologic test results in a population of blood donors entered into a multicenter, prospective study designed to evaluate the significance of HIV infection in the donor population. Three groups of donors were studied: those with confirmed HIV infection; age- sex- and race-matched controls, nonreactive in HIV ELISA; and ELISA-reactive donors with indeterminate blot patterns. We selected 98 individuals with indeterminate blot patterns from the latter two groups. On the basis of at least one, and up to five subsequent samples, drawn at 6-month intervals, we were able to establish that all but one of the 98 subjects had stable or transient indeterminate blot patterns and were, thus, not infected with HIV-1. The remaining individual, originally selected as a seronegative control subject, developed a blot pattern with weak bands at p55, p66, and gp160 on his second visit; this sample was nonreactive in ELISA tests for HIV antibody. At two subsequent visits, this subject had a full-positive Western blot pattern with reactions to all viral proteins. He had risk factors for HIV infection, and the subsequent samples were classified as a true positive.

The 98 samples were tested with two developmental ELISA procedures designed to detect antibodies to *env* glycoproteins (pENV9, DuPont, and Recombigen-EIA, Cambridge Bioscience). Both procedures are based on the use of a recombinant peptide that includes the junction between gp41 and gp120[18,19]; Recombigen also includes a recombinant p24. One of these tests has been proposed as a component of a diagnostic algorithm for HIV serology.[20] The patterns of reaction that we observed are outlined in Table 6. Although some samples were repeatedly reactive with one or other of the tests, there was no case in which the recombinant tests supported each other. These tests, in common with routine screening ELISAs, also failed to identify the single seroconversion sample described above, although they showed positive results on all subsequent samples and uniformly show positive results on samples that are confirmed to be positive. These data suggest that it may be possible to use additional serologic tests for antibodies to *env* glycoproteins in order to define[20]

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Table 6.—Reactivity of 98 Indeterminate Samples in Recombinant ELISA Tests for HIV-1 *env* Antibodies*

Test Results		
pENV 9	**Recombigen**	**No.**
+	−	2
−	+	6
+	+	0
−	−	90†

*Ninety-seven samples from individuals known to be uninfected on the basis of follow up; 1 early seroconversion. ELISA indicates enzyme-linked immunosorbent assay; HIV-1, human immunodeficiency virus type 1.

†This group included the early seroconversion sample, which was also nonreactive in licensed ELISA tests.

or exclude a diagnosis of HIV infection for most individuals with indeterminate blot patterns. In this issue of the ARCHIVES, Ascher and Wilber[21] point out that indirect immunofluorescence may also be effective in this context. More work to validate this approach to the resolution of blot patterns is in progress in this and other laboratories.

Although only a few cases of human immunodeficiency virus type 2 infection have been identified in the United States,[22] it is important to be aware that there is at least a theoretical possibility that some indeterminate patterns on HIV-1 Western blot tests could reflect infection with human immunodeficiency virus type 2. In particular, there is homology between the *gag* and *pol* genes of the two viruses[6] and, thus, HIV-1 blots with reactions to products of these genes may merit further investigation by the Centers for Disease Control.[13,23]

SUMMARY AND RECOMMENDATIONS

Screening tests for HIV antibodies do generate false-positive reactions and, when used to test populations with low prevalence rates, positive predictive values may be as low as 10%. Additional testing is necessary to confirm a reactive screening test, and the Western blot procedure is frequently used for this purpose. The Western blot is a technique for visualizing antibodies to individual viral polypeptides, and certain patterns of reactivity are interpreted as confirmation of HIV infection; the absence of any pattern signifies the absence of infection. Unfortunately, some indeterminate patterns occur that cannot be readily interpreted and require further resolution.

In using the Western blot procedure, the following recommendations should be considered.

1. The blot system used should be licensed, or should be shown to have equivalent performance characteristics to a licensed system.

2. Appropriate criteria should be used for evaluation of the blot. We believe that the criteria recommended by the ASTPHLD and CDC[14] are appropriate when additional diagnostic information is available or when used for epidemiologic surveys that do not involve notification of the subject. In circumstances where individuals are to be notified solely on the basis of serologic test results, we support the use of a three-gene criterion.[8,14]

3. Indeterminate Western blot results should be resolved. Currently, evaluation of a second sample, taken after 2 or more months, is most appropriate. However, it is anticipated that additional serologic analysis of the first sample may prove to be a satisfactory means of resolution of indeterminate blots.

4. The remote possibility that unusual blot patterns, particularly those characterized by the presence of reactions to both *gag* and *pol* gene products, may signify infection with retroviruses other than HIV-1 should be considered; further evaluation of the sample or patient may be indicated in a limited number of cases.[23]

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Immunofluorescence for Serodiagnosis of Retrovirus Infection

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• Indirect immunofluorescence antibody testing is a mainstay of retrovirus serodiagnosis in the public health community, affording quick, inexpensive, and clear results with significant advantages over Western blot testing. Limitations regarding training of personnel and availability of reagents will probably continue and limit indirect immunofluorescence antibody testing to the present users.

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Indirect immunofluorescence antibody (IFA) testing has been applied to the serodiagnosis of human retrovirus infection since the first characterization of these viruses.[1] Indirect immunofluorescence antibody testing affords the user an opportunity to visualize quickly a result and compare it with a built-in control. In contrast with Western blot analysis, it does not differentiate the component of the virus to which the serologic response is directed. Although IFA was used extensively in early studies, it has been supplanted by the Western blot technique for large-scale use in determining human immunodeficency virus (HIV) antibody status based on regulatory issues and the fact that the Western blot procedure is perceived to be the "gold standard" based on studies of the molecular biology of HIV. Although IFA has been overlooked in this evolutionary process, in our view it has an important, continuing role in retrovirus serologic investigations. The purpose of this review is to provide an annotated bibliography of the history of the immunofluorescence serologic investigation for retroviruses and discuss its current role and status.

HISTORY

After the description of the acquired immunodeficiency syndrome (AIDS) on clinical grounds, studies using IFA serologic techniques implicated the only known human retrovirus, human T-cell lymphotropic virus type I, in its origins. Using the difficult-to-interpret membrane fluorescence technique, Essex et al[2] claimed presence of antibody to human T-cell lymphotropic virus type I in approximately 25% of AIDS cases. Since it was later learned that there is neither a significant cross-reaction between human T-cell lymphotropic virus type I and human HIV type 1 nor a large percentage of doubly infected individuals, this study generated distrust within the scientific community of the IFA procedure in general for use in AIDS. Shortly after the discovery of HIV, IFA was applied by Kitchen et al[3] with a 70% overall success rate in clinical AIDS and good correspondence between IFA and radioimmunoprecipitation. Kaminsky and coworkers[4] adapted their AIDS–associated retrovirus isolate to this purpose in 1985 and published information on the variable nature of the end point titer obtained. The earliest work from Great Britain investigating antibodies to HIV, using both lymphadenopathy-associated virus and human T-cell lymphotropic virus type III strains in IFA, showed equivalence with an early radioimmunoassay in 95% of cases. The Public Health unit in England used IFA with fixed cells in a retrospective survey documenting the arrival of HIV infection in 1980 in their study population,[6] and Jones et al[7] used IFA in a similar retrospective study of hemophiliacs. Correspondence between these results using IFA and immunoperoxidase was reported by Karpas et al in 1985.[8]

Shortly after the licensing of the enzyme immunoassay (EIA) test kits for screening blood in the United States, a direct comparison was published between IFA and Western blot analyses for the confirmation of reactive blood donor samples.[9] A group using a locally developed EIA for screening claimed

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equivalence in reliability between IFA and Western blot analyses in confirming high-risk samples.[10] Ragni and collaborators[11] published findings showing that IFA-positive hemophiliacs had a high frequency of antibody to p24 or gp41 on Western blot investigations; the first prospective study of hemophiliac seroconversion used IFA.[12] Hedenskog et al[13] published an extensive study of the performance characteristics of IFA used as a sole test for HIV. Our laboratory compared antibody detection by EIA, IFA, and Western blot techniques and found IFA and Western blot techniques to be equivalent and also noted that IFA tests could actually detect antibody to HIV earlier than could EIA tests in a high-risk, public health clinic population.[14] Further confirmation of this phenomenon was published by Bach and Boothby[15] who presented results of an individual with AIDS dementia whose tests showed positive IFA and radioimmunoprecipitation results but negative EIA findings. These findings led to the improvement and relicensing of EIA reagents. Other comparative studies have been published with favorable results for the IFA method.[16,17] In a study by Carlson et al,[18] which otherwise showed equivalence between Western blot and IFA testing, IFA negative specimens that showed a "p24-only" Western blot analysis were considered positive, and the sensitivity of the IFA test was questioned. These specimens would now be interpreted as indeterminate (and probably negative).

The importance of the method of fixation of the cells has been pointed out by Fauvel and Ozanne.[19] Adaptation of the IFA method to advanced technology with laser light sources and flow quantitation has been reported.[20-22] Successful application of IFA in a commercial reference laboratory was reported by Lennette et al[23] and in general use in Belgium by van der Groen and coworkers[24] and in West Germany by Schmidt et al.[25] Although not published, the method is used internationally in reference laboratories ranging from Brazil to South Africa.

CURRENT STATUS

Indirect immunofluorescence antibody testing has specific utility in the diagnosis of retroviral infections in man. Using an infected cell substrate with the appropriate controls and well-trained personnel, the technique can be extremely useful for confirming screened EIA-reactive specimens. In a high-risk population, a clear positive result can often be obtained and reported at low cost and with great speed. A specimen with an indeterminate Western blot pattern can be reported as positive based on a reactive IFA. A negative IFA result on an EIA-positive specimen is not considered definitive and must be followed by a blot showing negative results or recombinant assay to result in an overall negative report. The sequence used in our laboratory, IFA as primary confirmatory test, followed by a Western blot test only for the resolution of nonspecific patterns, is the most efficient and cost-effective means of performing reportable HIV serologic findings in a population with any appreciable prevalence.

The Consensus Conferences on Testing for Human Retroviruses, sponsored by the Association of State and Territorial Public Health Laboratory Directors, have taken a strong stand on the use of IFA, including its endorsement as an alternative to Western blot testing in 1986.[26] Subsequent Consensus Conference statements have maintained that position, with the recommendation of a standard method,[27] a recommendation that the US Food and Drug Administration expedite approval,[28] and defining the role for IFA in the overall testing plan.[29] Through the use of these guidelines, the public health community has functionally adopted the IFA method.

Based on performance considerations alone, a strong case can be made for the use of IFA testing in retrovirus serodiagnosis in high-prevalence situations (other than blood banks). Additionally, using Western blot testing for confirming all HIV type 1 EIA-positive findings is prohibitively expensive and results in confusion due to indeterminate blot patterns in infected individuals. As an impediment to widespread use, IFA is a subjective procedure requiring training of personnel, and the performance of IFA by untrained personnel is not recommended. Licensing of reagents does not solve this problem. The model that exists at present, where major public health laboratories, both in California and around the United States use unlicensed immunofluorescence test procedures and cooperate in training personnel, appears to be the best compromise and should continue to be successful.

AVAILABILITY OF REAGENTS

In spite of the de facto approval described above, the field of immunofluorescence testing for retroviruses is stymied by the lack of availability of reagents approved by the US Food and Drug Administration. In the current climate of Food and Drug Administration approval driven by blood bank requirements, it is unlikely that an immunofluorescence assay can overcome its image as a subjective test and pass quality control requirements for approval. However, for use in public health settings such reagents should certainly be available and an alternative pathway, such as a device approval under the 510K process (ie, the form used in filing a product application for diagnostic use without the elaborate review required by biologic for all AIDS-related products), should be utilized. In any case, research reagents provided either by manufacturers or through the public health network as a cooperative venture will continue to cover the low-level requirements that such reagents represent.

OTHER RETROVIRUSES

As one would infer from the experience with HIV, the utility of IFA should be as great or greater in the case of serodiagnosis of infection with human T-cell lymphotropic virus types I and II, since the experience with confirmatory assays for those viruses is even more confusing. In our laboratory, IFA has been a mainstay of the epidemiologic studies conducted for a virus that is presumed to be like the human T-cell lymphotropic virus type II seen in drug users in California.[30] Other workers have compared IFA with Western blot and EIA analyses[31] and with particle agglutination[32,33] with favorable results. One article has appeared on the use of IFA in the diagnosis of infection with human T-cell lymphotropic virus type II[34] and we have used the procedure in serosurveys for this variant virus.

COMMENT

Indirect immunofluorescence antibody testing is a mainstay of retrovirus serodiagnosis in the public health community, affording quick, inexpensive, and clear results with significant advantages over Western blot testing. Limitations regarding training of personnel and availability of reagents restrict its use, and such restrictions will probably continue and limit IFA testing to the present users.

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Human Immunodeficiency Virus Type 1 Antigen and Culture Assays

J. Brooks Jackson, MD

• Commercial enzyme immunoassays have recently been developed for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) viral proteins in blood and other body fluids of infected patients. At the same time, culture assays for HIV-1 have become more rapid, sensitive, and specific for HIV-1 isolation from the blood of infected patients. Both of these assays have clinical utility in the diagnosis, prognosis, and monitoring of HIV-1 infection in certain patients.

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Detection of antibodies to human immunodeficiency virus type 1 (HIV-1) in the serum of HIV-1–infected individuals has and will probably continue to be the most inexpensive, rapid, and sensitive method of diagnosing HIV-1 infection. However, the recent development of sensitive HIV-1 culture methods and the commercial availability of HIV-1 antigen detection assays have enhanced the laboratory's capabilities in terms of the diagnosis, prognosis, and research of HIV-1 infection. The intent of this article is to update a previous review[1] by describing (1) recent methods used in HIV-1 antigen and HIV-1 culture assays, (2) the sensitivity and specificity of these assays, and (3) the clinical and research applications of these tests.

HIV-1 ANTIGEN DETECTION ASSAYS

In 1985, McDougal et al[2] first described an HIV-1 antigen enzyme immunoassay (EIA) that was successful in detecting HIV-1 in culture supernatants. Previously, the detection of HIV-1 in cell culture was primarily dependent on detection of reverse transcriptase (RT) activity, which is a relatively tedious procedure. The HIV-1 antigen EIA, on the other hand, was not only technically easier but also as sensitive and more specific. Nevertheless, the initial HIV-1 antigen EIAs were not sensitive enough to detect HIV-1 directly in the blood or body fluids of HIV-1–infected individuals.

However, in 1986 commercial EIAs became available for the direct detection of primarily the HIV-1 p24 core antigen in serum, plasma, and cerebrospinal fluid (CSF).[3] The principle of HIV-1 antigen detection in these kits involves incubating the patient's specimen for 1 hour or as long as overnight with a polystyrene bead or in a plastic microwell coated with monoclonal or polyclonal anti-HIV antibody. The bead or microwell is then rinsed and incubated with an enzyme-conjugated monoclonal or polyclonal rabbit or goat antibody to HIV-1 antigen to form a sandwich. Some assays add an enzyme-conjugated antigoat or antirabbit antibody to the probe antibody to enhance sensitivity and specificity (Fig 1). After washing, the appropriate substrate can then be added. If the antigen-antibody-enzyme complex is present, a color change will be detected by an increase in absorbance at a specific wavelength. Dilutions of known amounts of HIV-1 antigen can be used to generate a standard curve for quantitating the amount of free HIV-1 antigen in the patient's specimen. The detectable limit for this assay has been reported to be between 10 and 50 pg of HIV-1 antigen per milliliter depending on the assay and whether it detects only the p24 antigen or additional HIV-1 antigens.[4-6]

Because different kits may detect one or more HIV-1 antigens as well as use different reference material to generate a standard curve for quantitation, it is not surprising to find 1- to 10-fold differences in the level of detectable HIV-1 antigen in the same serum specimen using kits from different manufacturers.[5,6]

If a specimen is found to be repeatedly reactive, a specific antibody neutralization assay should be performed to confirm the presence of HIV-1 antigen. This assay is also commercially available with most HIV-1 antigen kits and involves preincubating the specimen or control with human antibody to HIV-1 before running the HIV-1 antigen EIA assay. If HIV-1 antigen is present, the neutralizing antibody will form an immune complex that prevents binding of the HIV-1 antigen to the HIV-1 antibody–coated bead or microwell. This results in a reduction of absorbance when compared with the specimen in which human serum nonreactive for antibody to HIV-1 is used in place of neutralizing antibody. A specimen is considered to be confirmed positive for HIV-1 antigen if the reduction in absorbance of the neutralized specimen when compared with a matched nonneutralized control is 50% or greater, and if the nonneutralized control absorbance is greater than or equal to the calculated cutoff.[6,7]

Serum, plasma, CSF, and cell culture supernatant have been shown to be suitable specimens for HIV-1 antigen detection by EIA. However, testing of lipemic or hemolyzed specimens should be avoided. If specimens are to be stored, they may be refrigerated at 2°C to 8°C for as long as 1 week or also frozen. Multiple freeze-thaw cycles of the specimen should be avoided.

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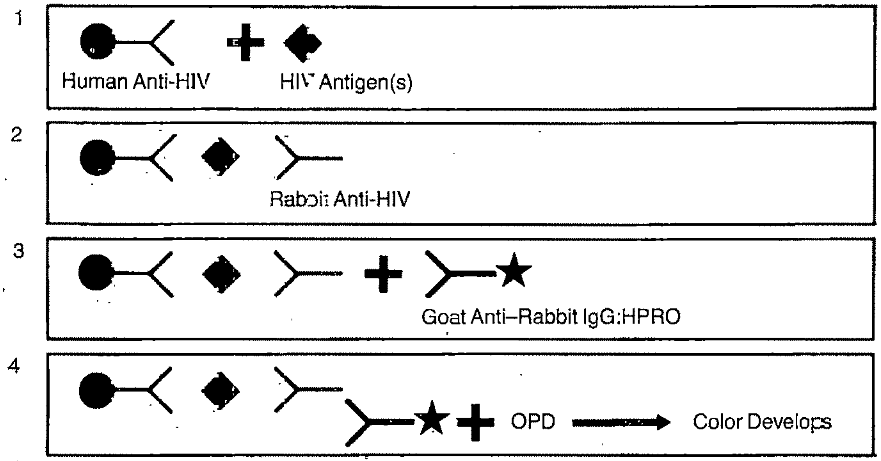

Fig 1.—Diagram of human immunodeficiency virus (HIV) antigen enzyme immunoassay design. HPRO indicates horseradish peroxidase; OPD, o-phenylenediamine. Sensitivity is 50 pg/mL of purified HIV. (Courtesy of Abbott Laboratories, North Chicago, Ill.)

The assay generally takes 4 to 24 hours to perform the entire procedure depending on the kit. If a test is repeated and then the specimen neutralized for confirmation, it may take 2 to 4 days to generate a positive test result. The cost of reagents for research laboratories is approximately $4 to $8 per test, so that obtaining a confirmed positive test result may cost at least $20 for reagents alone.

While the primary antibody used to capture the HIV-1 antigen is polyclonal in most assays the specific antibody for the p24 core proteins appears to have 10 000 times more affinity than the antibodies for gp120/41 envelope proteins.[8] Consequently, the current antigen EIA kits primarily detect p24 antigen. It is important to emphasize that these tests appear to detect free p24 antigen. In patients who have detectable levels of p24 antibody, p24 antigen appears to be complexed with antibody and is often difficult to detect.[3] This concept is also supported by a study in which 35 (70%) of 50 patients with Western blot–positive cultures with absent p24 antibody levels had detectable HIV-1 antigen, compared with 3 (8%) of 38 patients with detectable levels of p24 antibody.[10] Moreover, patients who are initially antigen positive after exposure often become antigen negative when they seroconvert and develop p24 antibody.[3] Likewise, it is common for patients to become antigen positive as their p24 antibody level falls during progression from the asymptomatic state to acquired immunodeficiency syndrome (AIDS).[8,10-12] It is not clear whether this increased positivity is due to greater antigen production as the disease progresses or solely due to falling antibody titers or both. One study suggests that the production of detectable HIV-1 antigen reflects enhanced expression of viral genes and that loss of p24 antibody is secondary.[10]

It should be noted that the ability of these assays to detect HIV-2 antigen is limited, judging from one study in which HIV-2 antigen could be detected in the serum of only 2 (11%) of 18 patients with HIV-2 infection using an HIV-1 antigen kit.[13]

The ability to detect the presence of HIV-1 directly in serum or CSF is a significant improvement over antibody testing in several respects. First, the direct detection of HIV-1 antigen indicates active HIV-1 infection as opposed to past exposure to HIV-1 proteins. Second, serum antigen detection may allow for the diagnosis of HIV-1 infection before antibody seroconversion. For example, in the Multicenter AIDS Cohort Study, 13 (8%) of 154 men known to have seroconverted were antigenemic 6 months before the visit at which antibody was first detected.[14] Third, the detection of HIV-1 antigen in serum has prognostic significance. Among HIV-1 antigen–positive, asymptomatic, antibody-positive gay men, 59% progressed to AIDS over a 36-month period compared with 15% of HIV-1 antigen–negative, asymptomatic, antibody-positive gay men.[11] Moreover, HIV-1 antigen levels tend to increase with disease progression in a given individual.[12] However, there appears to be no correlation between the level of HIV-1 antigen and the clinical stage of disease when comparing different individuals. For example, in our laboratory the mean HIV-1 antigen level of 22 antigen-positive asymptomatic individuals was 427 pg/mL (range, 23 to 1594 pg/mL), compared with 257 pg/mL (range, 58 to 903 pg/mL) in 21 antigen-positive patients with AIDS not receiving anti-HIV therapy.

Nevertheless, the sensitivity of the HIV-1 serum antigen assay is poor if one assumes that all HIV-1 antibody–positive individuals are infected other than those with maternal antibody or passive recipients of such immunoglobulins. The sensitivity of the test improves, with clinical progression of disease showing 19% positivity in asymptomatic patients, 46% in patients with AIDS-related complex, and 69% in patients with AIDS.[7] Likewise, the CSF of 5 of 9 adults with AIDS-related encephalopathy and 5 of 5 children with AIDS had detectable HIV-1 antigen.[3] However, none was detected in the CSF of 13 asymptomatic HIV-1 antibody–positive controls. The specificity is much better than the sensitivity. In testing serum of German blood donors, 462 (0.16%) of 295 360 samples were repeatedly reactive, but none could be neutralized, indicating false-positive reactions.[15]

While the HIV-1 antigen EIA is not an appropriate screening test owing to its poor sensitivity, there are several clinical situations in which it may be useful.

Individuals in High-Risk Groups Before Antibody Seroconversion

It is not known what percentage of individuals overall will express HIV-1 antigen in serum during the interval between exposure and seroconversion. One small study found that 10 (53%) of 19 patients had detectable HIV-1 serum antigen 11 weeks before seroconversion.[16] However, the sensitivity of commercial HIV-1 EIA screening tests for HIV-1 antibody has continued to increase, and therefore the period between detectable antigen when present and the detection of HIV-1 antibody is probably shorter now. Human immunodeficiency virus type 1 antigenemia has also been temporally linked to acute HIV-1 infection before the appearance of HIV-1 antibody.[17,18] This test may also be helpful if positive in an HIV-1 antibody–negative pregnant woman in a high-risk group for HIV-1 infection.

HIV-1 Antibody–Positive Asymptomatic Individuals for Prognostic Purposes

As noted above, asymptomatic individuals who were serum antigen positive were four times more likely to progress to AIDS over a 36-month period. Such patients could be selected for clinical trials using drugs active against HIV-1 such as zidovudine (formerly known as azidothymidine).[5]

HIV-1–Infected Patients Receiving Antiviral Drug Therapy

Mean HIV-1 antigen levels have been shown to decrease significantly at least over the short term in patients treated with zidovudine compared with placebo-treated controls.[19,20] Therefore, monitoring of HIV-1 antigen levels may be useful in measuring the antiviral effect of drug therapy, although it is still not clear whether p24 antigen will prove to be a reliable surrogate marker of disease progression. If monitoring of HIV-1 antigen levels is desired, sequential specimens should be saved and batch tested using the same lot of assay to reduce interassay variability.

HIV-1 Antibody–Positive Infants

The HIV-1 antibody may be passively transferred in utero from HIV-1–infected mothers without apparent HIV-1 transmission to the infant in 67% to 76% of cases.[21-23] A positive HIV-1 antigen test on the infant's serum may be useful in confirming HIV-1 infection,[21,25] although it is not known whether noninfectious HIV-1 viral proteins can cross the placenta and persist in the infant's circulation. Moreover, passive transfer of maternal p24 antibody can bind p24 antigen if present and therefore not be detectable in the serum of an infected infant. The polymerase chain reaction assay for detection of HIV-1 DNA sequences may be more informative in this setting.

Differentiating AIDS From Primary Immunodeficiency Syndromes in HIV-1 Antibody–Negative Children

Since antibody response may be blunted or absent in children with congenital immunodeficiency syndromes, the diagnosis of AIDS may be missed if only HIV-1 antibody testing is performed.[26] Human immunodeficiency virus type 1 antibody–negative children with an immunodeficiency syndrome should be checked for HIV-1 antigen especially if they received a transfusion before antibody testing or the mother is HIV-1 antibody positive.

Individuals With Indeterminate Supplemental Antibody Tests

Most healthy individuals in low-risk groups with indeterminate Western blot test results are probably not infected,[27,28] but a negative antigen test may provide some reassurance, especially if the Western blot or radioimmunoprecipitation assay indicates the absence of antibodies to HIV-1 p24 antigen.[29] However, there are no confirmed data to support this application. If confirmatory testing is needed, HIV-1 cultures or polymerase chain reaction assays are probably more applicable in this context.

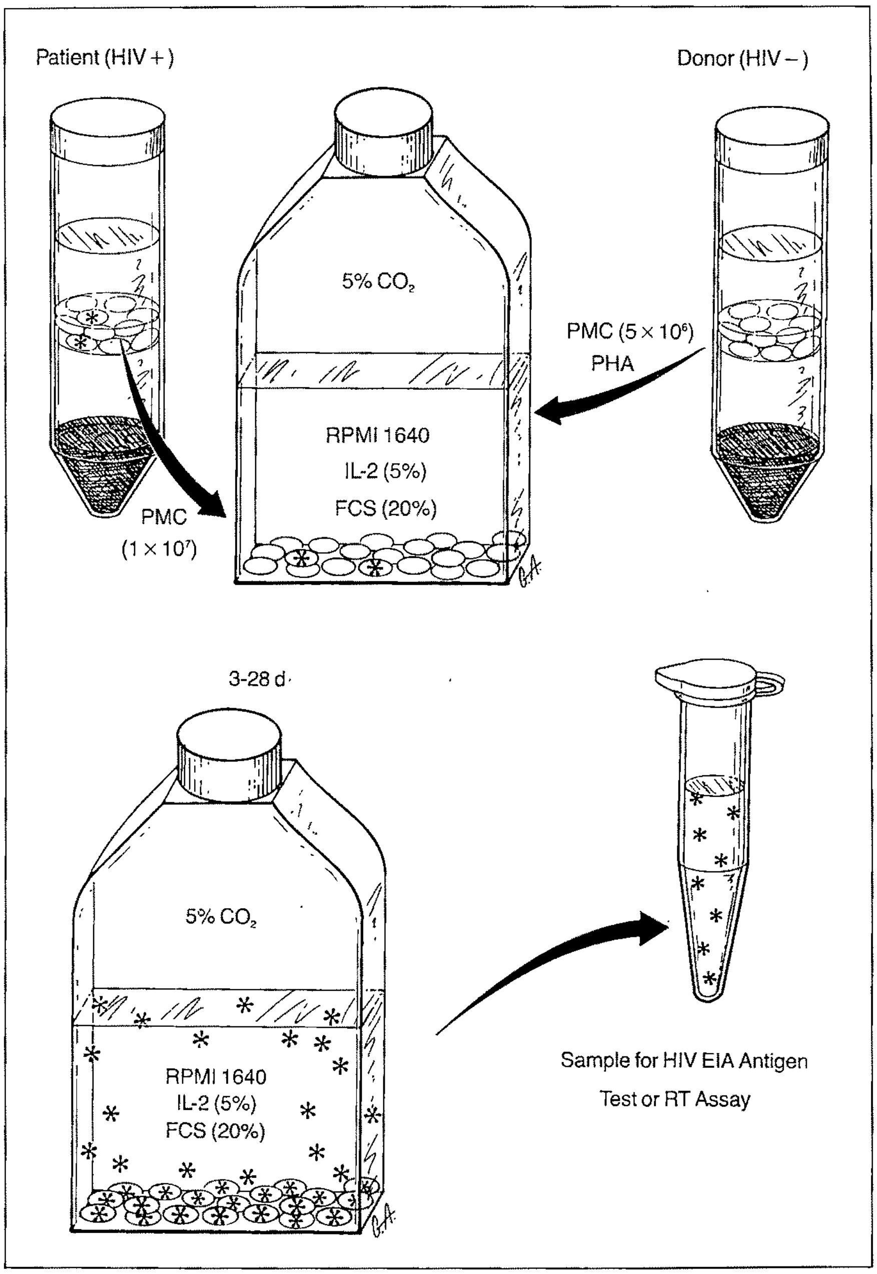

Fig 2.—Diagram of human immunodeficiency virus type 1 (HIV-1) culture assay. Plus indicates positive; minus, negative; PMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; IL-2, interleukin 2; FCS, fetal calf serum; RT, reverse transcriptase; EIA, enzyme immunoassay; and asterisks, HIV virus.

HIV-1 CULTURE ASSAYS

In 1983, HIV-1 was first propagated using phytohemagglutinin and T-cell growth factor in cocultures of lymph-node cells from a homosexual man with lymphadenopathy and T lymphocytes from a healthy adult donor and from umbilical-cord blood of newborn humans.[30] The process of HIV-1 was detected by (1) electron microscopy showing lymphocytes with characteristic immature particles with dense (C-type) budding at the plasma membrane; and (2) high levels of RT activity in the culture supernatant fluid on day 15 of coculture.

Subsequently, Gallo et al[31] and Popovic et al[32] confirmed these findings and developed a cell culture system using clones (H4, H9) of a neoplastic T-cell line (HT) for the continuous production of HIV-1. The presence of HIV-1 was further confirmed by serologic analysis[33,34] and by indirect immunofluorescence using fixed cells of HT-infected clones and rabbit antiserum to HIV-1 or patient serum.[22]

Levy and Shimabukuro[35] reported the recovery of HIV-1 by RT assay to be about 68% (53/78) in patients with AIDS, 41% (7/17) in HIV antibody–positive asymptomatic gay men, and 0% (0/85) in antibody-negative

HIV-1 Culture Method*

Patient PMCs are separated from 30 mL of heparinized blood on Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient within 24 h of collection. At this point, patient PMC can be directly placed in culture or slowly frozen at −190°C in RPMI-1640 medium containing 40% fetal calf serum and 10% dimethyl sulfoxide and cultured later after thawing and washing with equally good recovery.

1 × 10^7 PMCs are placed in 25-cm² tissue culture flask containing total of 15 mL of RPMI 1640 medium, 20% fetal calf serum, 5% interleukin 2, 5 mg/L of hexadimethrine bromide, 200 U/mL of penicillin, 200 mg/L of streptomycin, and 5 × 10^6 PHA-stimulated PMCs from HIV-1 antibody-negative blood donor. Donor PMCs are PHA stimulated (4 mg/L) for 2-4 d in above medium without interleukin 2 before addition to patient PMCs.

Cocultures are incubated at 37°C in 5% CO_2 atmosphere for up to 28 d.

Approximately 8 mL of culture medium above settled PMCs is removed twice per wk for HIV-1 antigen testing and replaced with equal volume of fresh medium. Additional 3 × 10^6 PHA-stimulated donor PMC are added once per wk.

*HIV-1 indicates human immunodeficiency virus type 1; PMCs, peripheral blood mononuclear cells; and PHA, phytohemagglutinin.

healthy heterosexuals using cocultures of patient peripheral mononuclear cells (PMCs) and phytohemagglutinin-stimulated human PMCs from clinically healthy individuals. In 64% of the positive cultures, RT activity was detected within 9 to 15 days from initiation of culture and in an additional 21% after 21 days. From a diagnostic viewpoint, these results were rather discouraging, because the sensitivity of culture is relatively poor, the time to positivity is relatively long, the RT assay is tedious, and the cost of reagents and labor is several hundred dollars. Moreover, magnesium ion–dependent RT activity is not specific for HIV-1 and may be produced by other retroviruses such as human T-cell lymphotropic virus type I.[36] Fortunately, refinements in HIV-1 cell culture techniques and the development of sensitive HIV-1 antigen detection EIAs in the last several years have dramatically improved the recovery rate of HIV-1 and decreased the time and cost of HIV-1 culture.[37] In our laboratory we use a rapid coculture technique to recover HIV-1 from the blood of infected patients (Table, Fig 2).

Using this cell culture method and one of the commercial HIV-1 antigen EIAs (Abbott Laboratories, North Chicago, Ill) described above, we have been able to detect the production of HIV-1 antigen in the culture supernatants of cocultivated PMCs from 57 (100%) of 57 patients with AIDS and 69 (99%) of 70 HIV-1 antibody–positive asymptomatic gay men.[37] In terms of time-to-positivity, HIV-1 antigen was detected in 46% of positive culture supernatant fluids by day 3 of culture, 94% by day 10 and 100% by day 21. Sixty percent of cultures of patients with AIDS were positive by day 3 of culture compared with 19% of those from asymptomatic seropositive patients, suggesting a greater viral load or more efficiently replicating strains in patients with AIDS.

In our laboratory, it appears that HIV-1 can replicate more quickly and consistently in mitogen-stimulated donor PMCs than in cell lines. However, it is necessary to refeed the culture every week or two with freshly stimulated PMCs to sustain HIV-1 replication.

In addition, the HIV-1 antigen EIA has the following advantages over the RT assay for detection of HIV-1 in culture: radioactive isotopes and a scintillation counter are not required; pelleting of supernatant with an ultracentrifuge or polyethylene glycol is not necessary; less sample volume (200 µL vs 1 to 3 mL) is needed; technical hands-on time is less (4 vs 5 hours); 5 to 10 times the number of samples can be tested per run; the cost of HIV-1 antigen EIA supplies per patient assay is approximately one fifth that of the RT assay; and the HIV-1 antigen EIA is more sensitive than RT in detecting HIV-1.[38,39] Moreover, the RT assay is much more difficult to standardize. Consequently, many laboratories have discontinued using it.

However, the RT assay has the theoretical advantage of detecting other HIV strains (HIV-2) or other retroviruses that the current HIV-1 antigen EIAs may miss.[36,40] There are a number of published RT assay procedures for those laboratories interested in detecting RT activity in concentrated culture supernatant fluid.[35,36,41-43]

The clinical utility of isolating HIV-1 from the PMCs of different patient groups is similar to that of the serum HIV-1 antigen assay. However, culture is more sensitive. In addition, several studies have reported a strong association between clinical status and the time-to-positivity in culture.[41,44,45] It is not clear whether time-to-positivity reflects a larger viral inoculum size (ie, viral load), or a highly replicative and fast-growing isolate or both. Time-to-positivity has been shown to increase from 7 to 22 days with a 10^{-4} dilution of stock virus,[46] suggesting that viral load may be responsible, but highly replicative strains are equally plausible. In addition, different donor cells for cocultivation may have different degrees of sensitivity to HIV-1. Therefore, time-to-positivity may not accurately reflect the virulence or viral load in the patient. Whether time-to-positivity will be a reliable predictor of progression to disease remains to be seen.

Human immunodeficiency virus type 1 culture may also be useful in monitoring drug therapy. The authors of one study reported a decreased ability to isolate HIV-1 from mononuclear cells in patients receiving an antiviral drug called ampligen.[47] However, others have seen no difference in HIV-1 isolation rates in patients treated with zidovudine vs placebo.[48] The drawbacks to HIV-1 culture are that it is expensive, may take weeks, and creates the potential for exposure of personnel to high concentrations of virus.

Human immunodeficiency virus type 1 can also be cultured from other body fluids including CSF,[49] serum,[50] cervical secretions,[51] saliva,[52,53] semen,[54,55] plasma,[56,57] tears,[58] breast milk,[59] and brain tissue.[60] However, the percentage of HIV-1 recovery is less from these fluids: 73% in CSF from patients with AIDS-related neurologic syndromes,[49] 4% in saliva of patients with AIDS,[53] and 70% in the plasma of patients with AIDS.[56] The clinical utility in culturing these fluids is not clear, although one study suggests that recovery of HIV-1 from CSF in patients with neurologic disorders indicates HIV-1 as the probable cause since HIV-1 was not recovered from CSF of patients with AIDS who did not have neurologic problems.[49] Another study suggests that virus isolation from plasma is more reliable than antigen detection for the demonstration of HIV-1 in primary infection before antibody seroconversion.[57]

CONCLUSIONS

As the AIDS epidemic spreads, the demand for HIV-1 testing will continue to grow. While HIV-1 antibody testing will most likely continue to be the diagnostic assay of choice for detection of HIV-1 infection, HIV-1 antigen and culture assays may be useful for diagnosis, prognosis, and drug evaluation in certain patients.

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Synthetic Peptide Assays to Detect Human Immunodeficiency Virus Types 1 and 2 in Seropositive Individuals

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• **Detection of antibodies to human immunodeficiency virus type 1 by enzyme-linked immunosorbent assay is the standard method to screen blood products. With the description of human immunodeficiency virus type 2 as a second retrovirus involved in the pathogenesis of acquired immunodeficiency syndrome, additional laboratory testing is required to screen blood products. However, owing to the serological cross-reactivity of human immunodeficiency virus types 1 and 2 at the molecular level, it is impossible to distinguish human immunodeficiency virus type 2–infected blood with enzyme-linked immunosorbent assays formatted with human immunodeficiency virus type 1 viral lysates. Synthetic peptides from the conserved envelope region of the human immunodeficiency virus transmembrane protein offer a new approach to develop site-specific diagnostic assays that will identify and discriminate between human immunodeficiency virus types 1 and 2.**

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The human immunodeficiency virus type 1 (HIV-1) was isolated in 1983.[1] This event facilitated the development of enzyme-linked immunosorbent assays (ELISAs) as the accepted method to screen blood products to prevent infection. These ELISAs were formatted with HIV-1 propagated in continuous cell lines with whole-virus lysates as antigens.[2,3] While the diagnostic efficacy of these assays has been established, a small percentage of false-positive results require laboratories to perform secondary tests such as the Western immunoblot to confirm spurious ELISA results.[4]

Recently, another human pathogenic retrovirus, termed HIV-2, has been isolated from patients with an acquired immunodeficiency syndrome–like syndrome in West Africa.[5] A single documented case of acquired immunodeficiency syndrome due to HIV-2 infection has been reported in the United States.[6] Confirmed HIV-2 cases have also been observed in Western Europe. The seroepidemiology of HIV infections and implications for public health have been recently summarized.[7]

Immunoassays for HIV need to be developed that satisfy two main criteria. (1) A simple test must be established that will distinguish HIV-1 and HIV-2 in locales where both viruses are endemic. Enzyme-linked immunosorbent assays where whole-virus lysates are used as the antigen source cannot effectively distinguish HIV-1 from HIV-2 owing to the cross-reactivity in highly conserved regions of the core and polymerase proteins of these viruses.[8] (2) Immunoassays must be available to laboratories involved in blood screening that are highly sensitive and specific. In this review article, evidence will be presented that synthetic peptides from immunoreactive domains of HIV-1 and HIV-2 viral proteins offer an approach to design immunoassays that will distinguish HIV-1 from HIV-2. Alternatively, these peptides can be used in combination to develop screening tests that can detect both HIV-1 and HIV-2 infected persons.

HIV VIRAL PROTEINS

Studies with HIV nucleic acid from various HIV isolates have revealed extensive heterogeneity.[9] There is approximately 60% homology between HIV-1 and HIV-2 in the core protein (*gag*) and 42% in the envelope protein (*env*).[10] The *env* region of HIV-1 contains two proteins, a 120 000-kd glycoprotein (gp120) and a 41 000-kd trans-

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membrane glycoprotein (gp41). Serological studies have revealed significant cross-reactivity between the two viruses in the core and polymerase proteins.[5] This observation precludes the use of HIV-1 ELISAs with whole-viral lysates as a method of determining whether an individual is infected with HIV-1 or HIV-2. However, standard ELISAs and Western immunoblotting can often detect infection with either virus with clinical serum samples, despite the average of about 50% homology between the two viruses.[8,11,12]

SYNTHETIC PEPTIDE TECHNOLOGY

A synthetic peptide can be used as an immunogen (to generate peptide-specific antibodies), as a vaccine, or as an antigen (to detect antibody responses in clinical serum samples). The solid-phase chemistry to produce these unique reagents has recently been reviewed.[13]

Viral proteins usually contain multiple antigenic sites. An antigenic site or epitope can either be a linear sequence of amino acid residues, or determinants that are dependent on the three-dimensional structure of the protein. The challenge for the investigator is to predict which epitopes have the highest probability of being a B-cell determinant. Criteria for the selection of candidate epitopes have been reviewed.[14] Regions from viral proteins should be examined that are likely to be exposed on the surface of the protein molecule. Peptide sequences should be selected that include elevated hydrophilicity,[15] β turn potential,[16] flexibility, and sequential mobility.[17,18] These variables unique to protein structure have been coordinated into computer programs to predict antigenic determinants.[19] While computer programs are useful in predicting immunodominant epitopes, these predictions are not 100% accurate. The alternative is to synthesize sequential overlapping peptides in a region of interest.

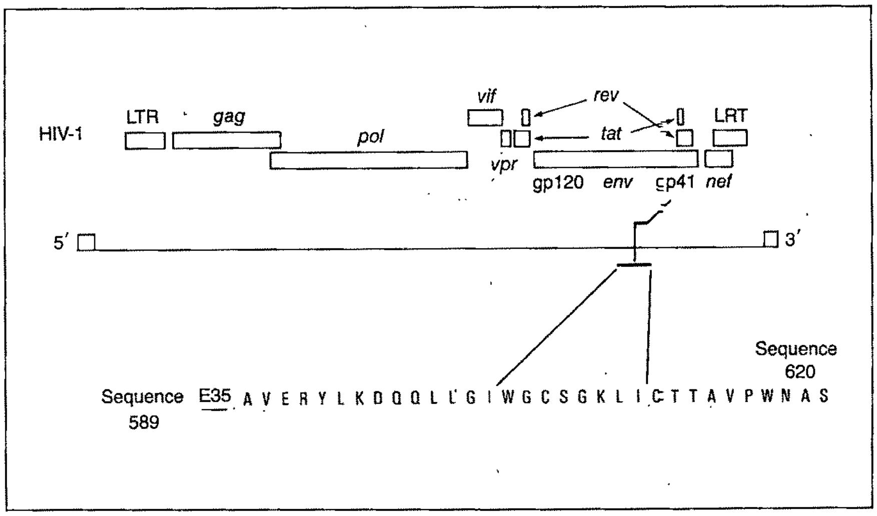

Fig 1.—Sequence and genetic location of human immunodeficiency virus type 1 envelope (*env*) synthetic peptides E32, E34, and E35. These peptides were derived from a conserved sequence of gp41 of human immunodeficiency virus type 1. *gag* indicates core protein; *pol*, polymerase; LTR, long-terminal repeat; *vif*, virion infectivity factor (R); *vpr*, (R); *rev*, regulator of expression of virion protein; *tat*, transactivator; *env*, envelope; and *nef*, negative factor.

IMMUNOREACTIVE EPITOPES OF HIV-1

Several investigators have synthesized peptides from computer-predicted epitopes of HIV with limited success. From these studies, one epitope was identified at the carboxyl terminus of HIV-1 gp120 and contains amino acids 504 to 518.[20] A second epitope, amino acids 735 to 752, has been described near the carboxyl end of gp41.[21] However, these HIV-1 *env* peptides were not reactive with a majority of HIV-positive serum samples. Parenthetically, evidence from recombinant HIV *env* proteins expressed in *Escherichia coli* suggested that the gp41 *env* region was highly immunoreactive.[22]

A well-characterized epitope of HIV-1 lies between *env* amino acids 584 to 618 near the amino terminus of gp41.[23-27] This region is moderately hydrophobic and is highly conserved in amino acids.

A series of related sequences were synthesized from the 584 to 620 region of HIV-1.[23] One peptide labeled E8 was found to be immunoreactive with human serum. The sequence for this peptide is as follows: I-W-G-C-S-G-K-L-I-C-T-T-A-V-P. From these studies, the epitope was further refined to include two oligopeptides, labeled E32 and E34. Another peptide labeled E35 was also synthesized that contained all the amino acid residues in the E32 and E34 peptides. The amino acid sequences for the E32, E34, and E35 peptides are shown in Fig 1. Subsequent studies indicated that the E32 and E34 peptides were very immunoreactive with clinical serum from a longitudinal study of homosexual men in San Diego, Calif.[24]

One of the cardinal features observed with the HIV-1 *env* synthetic peptide ELISA was the sharp dichotomy observed between an absorbance with positive sera compared with the absorbance of negative sera.[24] This distinction between positive and negative sera has been confirmed by other investigators with comparable synthetic peptides.[28] Assay results with the HIV *env* ELISA on 490 nonselected donor specimens and 493 HIV-seropositive sera are shown in Fig 2. In addition, the HIV *env* ELISA has been shown to detect the early rise of HIV-1 antibody in the seroconversion process.[24,28] Further refinement of the HIV-1 *env* epitope resulted in the data given in Table 1 assayed with the E35 peptide. A total of 5029 serum samples were entered into the study. These serum samples were assayed in the HIV-1 *env* ELISA compared with ELISA methods A and B, both licensed HIV-1 procedures. Assay results with the HIV-1 *env* ELISA resulted in 4 potential false-negative results and a calculated sensitivity of 99.8% in the HIV-1 positive sera. Conversely, assay results with ELISA methods A and B on similar study serum samples resulted in 7 and 5 potential false-negative results, respectively, with a 99.6% and 99.7% sensitivity for these two methods.[24] When 228 normal donor sera were assayed in the HIV-1 *env* peptide assay, a

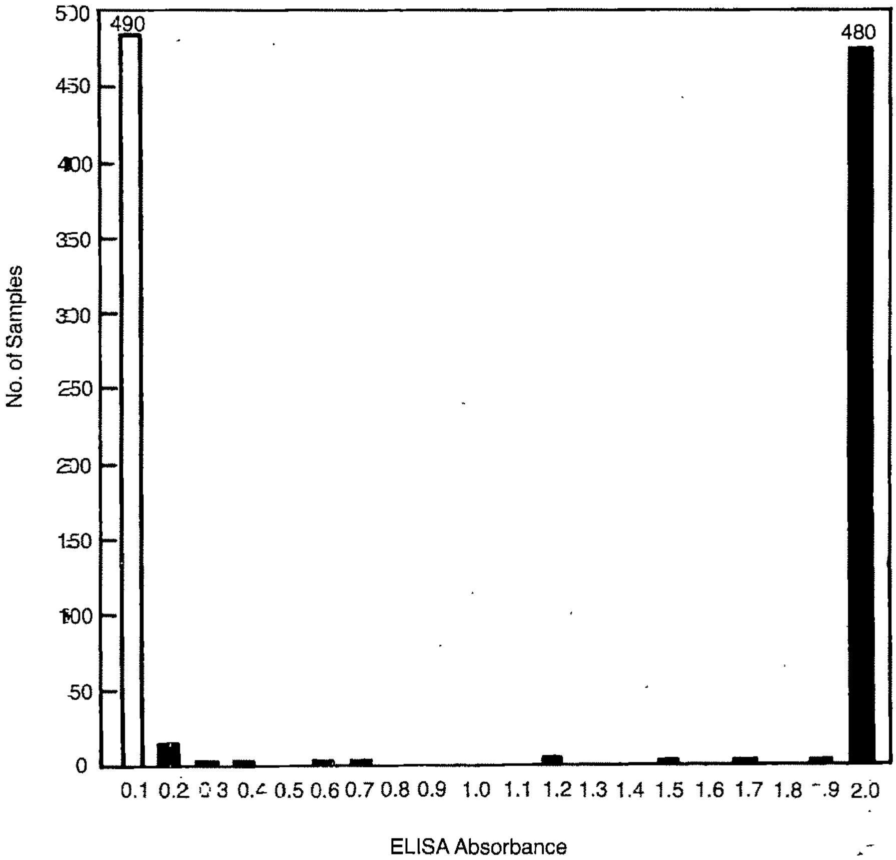

Fig 2.—Human immunodeficiency virus type 1 envelope (*env*) peptide data from a screen of 490 negative and 493 positive serum samples. The horizontal axis represents the absorbance at 490 nm. The height of the bar represents the number of samples at a given absorbance. The black bars indicate human immunodeficiency virus type 1 *env* peptide–positive samples; open bars, negative samples; and ELISA, enzyme-linked immunosorbent assay.

Table 1.—Sensitivity of HIV-1 Envelope Peptide ELISA Compared With Licensed HIV-1 ELISA Methods*

	HIV-1 Envelope ELISA (n=3029)	ELISA Method A (n=3029)	ELISA Method B (n=3029)
No. reported negative	1335	1286	1312
No. reported positive	1700	1729	1717
Potential false positives	4	7	5
Sensitivity, %	99.8	99.6	99.7

*HIV-1 indicates human immunodeficiency virus type 1; ELISA, enzyme-linked immunosorbent assay.

mean absorbance of 0.022 was observed. The cutoff for the peptide was established to be 0.150 for clinical studies or 6 SDs from the mean. The 228 donor sera were assayed by HIV-1 ELISA procedures A and B. The specificity for the peptide assay and ELISA method A was determined to be 100%. The specificity for ELISA method B was 98.4%.

HIV-2 *env* ELISA

The immunological cross-reactivity observed by the core *gag* proteins of HIV-1 and HIV-2 and the weak cross-reactivity observed in the *env* region of the two retroviruses allowed for the serological distinction between the two viruses.[29] Results from Western blot analysis of serum reactive for HIV-1 and HIV-2 indicated that there was a major epitope associated with the transmembrane glycoprotein and that this epitope could be used to discriminate HIV-1 and HIV-2 serum samples at the molecular level.[29] Synthetic peptides from the *env* region of HIV-2 and simian immunodeficiency virus (SIV) have been synthesized and found to be very immunoreactive.[30-33]

The sequences of these HIV-2 synthetic peptides used in these studies are shown in Fig 3. The HIV-1 sequence E35 is described in Fig 1. The HIV-2/SIV sequences are shown in Fig 3, with the divergent HIV-2 and SIV amino acids from the HIV-1 sequence italicized. An ELISA was established with synthetic peptides from the SIV *env* region and used to screen HIV antibody-positive serum and normal serum from North America and Africa. Representative specificity data with the HIV-2 ELISA are given in Table 2, with assay results from normal serum samples from North American–European and African populations. Cutoff criteria for the HIV-2 *env* ELISA were established as previously described.[24]

An absorbance greater than 0.2 was considered to be positive. A total of 1742 serum samples were assayed in the HIV-2 ELISA. Sixteen North American and European serum samples and 10 African serum samples were scored positive in the HIV-2 ELISA. The percent nonreactive was 97.9% for the North American–European normal group and 99.0% for the African normal group.

Data on the sensitivity of the HIV-1 *env* and HIV-2 *env* ELISAs when African serum samples were assayed are given in Table 3. These serum samples had been characterized by immunoassay with HIV-1 and HIV-2 ELISA procedures. A total of 243 specimens were assayed positive in the HIV-1 *env* ELISA and 251 specimens in the HIV-2 *env* ELISA. This is additional serological evidence that these two retroviruses are endemic in the African population and can be distinguished by the HIV-1 and HIV-2 *env* peptide ELISAs. The results in Table 4 suggest that there may be some cross-reactivity between the two HIV *env* ELISAs, or alternatively, the possibility exists of a dual infection in the African serum samples. When 759 serum samples were assayed in both ELISA procedures, 55 specimens were reactive in both protocols. This apparent cross-reactivity could be due to an expanded recognition of the HIV *env* epitope. The amino acids located at the N-terminal side (residues 587 to 595 of HIV-1) are highly conserved

HIV-1 A V E R Y L K D Q Q L L G I W G C S G K L I C T T A V P W N A S

HIV-2 A *I* E *K* Y L *Q* D Q *A* *R* L *N* *S* W G C *A* *F* *R* *Q* *V* C *H* T *T* V P W *V* *N* *D* S

SIV_{mac} A *I* E *K* Y L *E* D Q *A* *Q* L *N* *A* W G C *A* *F* *R* *Q* *V* C *H* T *T* V P W *P* *N* *A* S

Fig 3.—Conserved amino acid sequences of the envelope transmembrane glycoprotein of human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), and simian immunodeficiency virus (SIV_{mac}), used in the synthetic peptide enzyme-linked immunosorbent assays. The italicized residues are divergent residues from the HIV-1 sequence observed in HIV-2 and SIV_{mac}.

Table 2.—Specificity of HIV-2 Envelope Peptide Assay on Normal Serum Samples*

	North American and European (n=756)	African (n=986)	Total (n=1742)
No. scored negative	740	976	**1716**
No. of false positives	16	10	**26**
Nonreactive, %	97.9	99.0	98.5

*HIV-2 indicates human immunodeficiency virus type 2.

Table 3.—HIV-1 and HIV-2 Synthetic Peptide Assays on African Serum Samples*

	HIV-1 Assay (n = 933)	HIV-2 Assay (n = 1390)
No. negative	690	1139
No. positive for HIV-1	243	...
No. positive for HIV-2	...	251

*HIV-1 and HIV-2 indicate human immunodeficiency virus types 1 and 2.

between HIV-1 and HIV-2. This conservation could explain the cross-reactivity that we and others have observed.[34]

These results indicate that with site-specific synthetic peptides from the *env* region of HIV-1 and HIV-2, ELISA can be used to discriminate acquired immunodeficiency syndrome due to HIV-1 and HIV-2. The small percentage of cross-reactive samples observed in these two tests can be resolved with further refinement of the HIV-2 synthetic peptide.

Table 4.—HIV-1 and HIV-2 Synthetic Peptide Assays in African Serum Samples Assayed in Both Protocols*

	HIV-1 Assay (n = 759)	HIV-2 Assay (n = 759)	Both Assays (n = 759)
No. negative	538	620	...
No. positive for HIV-1	221	...	...
No. positive for HIV-2	...	139	...
No. positive in both protocols	...	...	55

*HIV-1 and HIV-2 indicate human immunodeficiency virus types 1 and 2.

CONCLUSION

Studies with synthetic peptides from the *env* region of the HIV transmembrane region have identified an immunodominant epitope that is reactive with serum samples from virtually all HIV-infected individuals. The advantages of synthetic peptide include the use of a chemically defined reagent (epitope) in ELISAs. These chemically synthesized epitopes contain few if any contaminants. This is in contrast to viral lysates or biological contaminants associated with recombinant protein from expression vectors. The HIV *env* peptides represent a single epitope that elicits an antibody response in nearly all infected individuals. Therefore, sensitivity of these ELISAs is comparable with licensed HIV procedures with viral lysates as antigens. Conversely, false-positive results have been reported with conventional ELISAs for HIV. The HIV *env* ELISA has the advantage of enhanced specificity owing to the nature of the chemically defined epitope, and the relative concentration of the epitope on the solid phase can be manipulated. Finally, there are no safety problems inherent in large-scale production of peptides, as there can be with viral lysates for HIV.

The use of a single-epitope assay has allowed for the simultaneous detection of antibody to HIV and the detection of surface antigen of hepatitis B.[35] This assay in a one-microwell format can reduce workload in a blood bank environment and cannot be performed with assays formatted with viral lysates or recombinant proteins.

The exquisite specificity of the HIV *env* peptides described in this review will allow progress to characterize assays that will distinguish patients with HIV-1 and HIV-2 infections. An additional advantage of synthetic peptides will be the latitude to develop other assay formats to simplify and expand HIV testing in endemic areas.

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In Other AMA Journals

ARCHIVES OF SURGERY

Elevated Tumor Necrosis Factor Production Concomitant to Elevated Prostaglandin E2 Production by Trauma Patients' Monocytes

Thomas K. Takayama, MD; Carol Miller, PhD; Gyorgyi Szabo, MD (Arch *Surg*. 1990;125:29-35)

Accelerated Lung Amino Acid Release in Hyperdynamic Septic Surgical Patients

Donald A. Plumley, MD; Wiley W. Scuba, MD, ScD; R. Ken Hautamaki, MD; Tomas D. Martin, MD; Timothy C. Flynn, MD; W. Robert Rout, MD Edward M. Copeland III, MD (*Arch Surg*. 1990;125:57-61)

Identification of a Novel Tumor Necrosis Factor Cachectin From the Livers of Burned and Infected Rats

Christopher Keogh; Yuman Fong, MD; Michael A. Marano, MD; Sophie Seniuk; Wei He, MD; Annabel Barber, MD; Joseph P. Minei, MD; Diane Felsen, PhD; Stephen F. Lowry, MD; Lyle L. Moldawer, PhD (*Arch Surg*. 1990;125:79-85)

Detection of Human Immunodeficiency Viruses by the Polymerase Chain Reaction

John J. Sninsky, PhD, Shirley Kwok

• The human immunodeficiency viruses types 1 and 2 have been implicated as the etiologic agent of the acquired immunodeficiency syndrome and its related disorders. The direct detection of human immunodeficiency virus is complicated by the low incidence of free circulating virus as well as the small number of infected cells. An in vitro DNA amplification procedure known as the polymerase chain reaction has been applied to the detection of the human immunodeficiency virus proviral sequences in infected individuals. This article highlights the features of the polymerase chain reaction and its contribution to the detection of these viruses.

(*Arch Pathol Lab Med.* 1990;114: 259-262)

Two human retroviruses of the Lentivirinae subfamily have been associated with acquired immunodeficiency syndrome. The viruses, human immunodeficiency viruses (HIV) types 1[1,2] and 2[3,4] share approximately 60% overall nucleic acid sequence homology for the more conserved *gag* and *pol* genes and from 30% to 40% for the other viral genes and long terminal repeat.[3] Human immunodeficiency virus 1 is the recognized agent of acquired immunodeficiency syndrome in the United States, Europe, and Central Africa, while HIV-2 is prevalent primarily in West Africa. The preliminary suggestion that HIV-2 is less pathogenic[5] merits further study.

The HIV life cycle[6] begins by attachment of virus particles to cells via CD4 receptors present most notably on mononuclear cells of T4 and macrophage lineages. On entry into the cell, the virus uncoats, and a DNA copy of the RNA genome (provirus) is synthesized by the viral encoded reverse transcriptase. The DNA can either circularize and integrate into the host genome or remain unintegrated in the cytoplasm. Human immunodeficiency virus replication is restricted at this stage until the infected cell is activated. The in vivo activation of HIV replication is not clearly understood, but in vitro activation can be accomplished by various agents, including interleukin 2[2] 5-iodo-2, deoxyuridine,[7] hydrocortisones and other hormones,[8] and tumor necrosis factor.[9] Spliced messenger RNAs encode multiple proteins involved in translational and transcriptional regulation of various stages of the viral life cycle; unspliced messenger RNAs encode the structural proteins of the virus. Posttranslational processing of viral structural polyproteins and assembly with genomic RNA at the cell surface results in the budding of mature virions.

The detection of antibodies to the viral proteins has played a significant role in the identification of individuals who are infected with these viruses. However, in many situations a direct detection of the virus would be desirable. Direct detection of HIV has proved refractory because of the small number of cells harboring HIV, the small number of proviral copies per infected cell, and the transcriptional dormancy of the viral genome.

Several procedures have been developed for detection of HIV. Of these, virus culturing has been the standard. However, even with cocultivation the successful isolation of virus from seropositive individuals and those with documented infection varies widely.[10] Moreover, the procedures are labor intensive, time-consuming, and costly. The use of in situ hybridization to directly detect viral nucleic acid in transcriptionally active cells[11] and Southern blot analysis of HIV DNA[12] extracted from lymphocytes has only been successful from a fraction of infected individuals. On the other hand, DNA isolation from lymph node and brain[13] has led to a higher, but still inadequate, proportion of individuals positive for HIV sequences by these procedures. The identification of viral p24 nucleocapsid antigens by specific polyclonal or monoclonal antibodies also suffers from the lack of sensitivity.[14] The sequestering of viral antigen in circulating immune complexes compromises this approach. Further-

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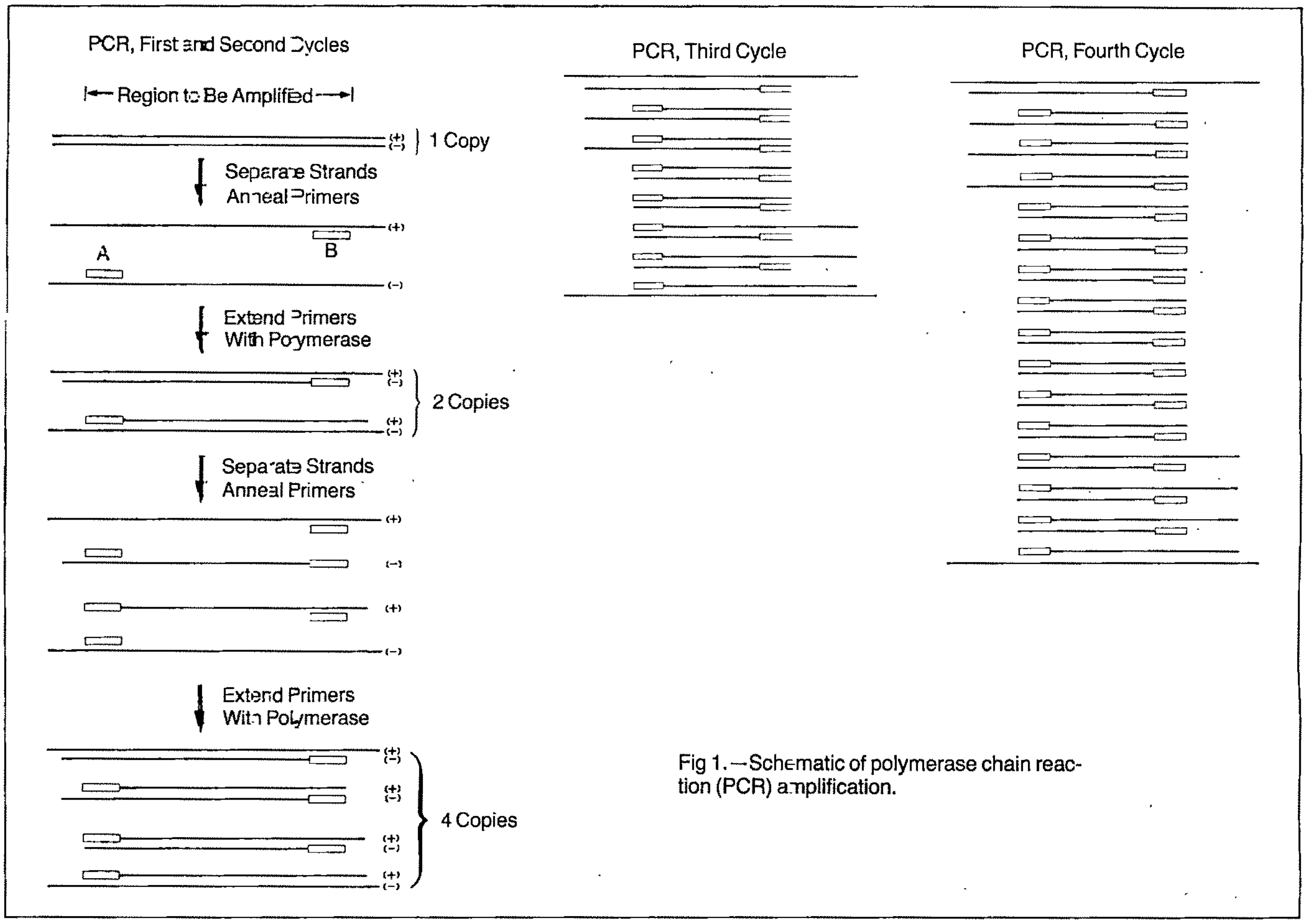

Fig 1.—Schematic of polymerase chain reaction (PCR) amplification.

more, antigens may not be detected during the latent stage when virus has established infection, but is not actively replicating.

An in vitro DNA amplification procedure, termed the *polymerase chain reaction* (PCR),[15,16] (Fig 1) has been successfully applied to the sensitive and specific detection of HIV proviral DNA. The use of a thermostable polymerase has further enhanced the application of PCR.[17] The procedure uses two synthetic oligonucleotides (primers) that are complementary to sequences that flank a region of interest; the upstream primer is complementary to the negative strand DNA and the downstream primer is complementary to the positive strand. Each cycle of PCR consists of a three-step process: denaturation of the DNA duplex, annealing of the primers, and extension of the primers with a polymerase in the presence of deoxynucleoside triphosphates. Theoretically, each cycle of PCR results in a doubling of the targeted sequence so that, for example, after 20 cycles of amplification a million copies of the DNA fragment are generated from a single copy. The procedure has also been applied to RNA by first transcribing the RNA to complementary DNA with reverse transcriptase and then amplifying the complementary DNA by the standard procedure.

The amplification of proviral DNA enables detection of not only cells actively replicating virus, but also those that harbor quiescent provirus. Although active HIV transcription can potentially generate a large number of transcripts, the limited number of studies exploiting RNA as a template[18,19] to date, suggest that more sensitive detection can be obtained if DNA is used directly as a template for PCR.[19] A productive area of research is the use of PCR to monitor and compare the messenger RNAs from the structural and regulatory genes.

Sequence variation will affect the stability of the primer-template duplex and, consequently, extension by the polymerase. Given the extensive heterogeneity of the viral genome,[12,20-23] it was necessary to amplify only highly conserved regions so as to provide detection of all viral variants both within and between infected individuals. The availability of nucleic acid alignments of all sequenced HIV isolates (Human Retroviruses and AIDS, Los Alamos [NM] National Laboratory, 1989) expedited the identification of conserved regions.

The commonly used primer-probe systems and reaction conditions for HIV amplification and detection have been described.[24,25] It is important to note that the reaction parameters must be optimized for each primer-pair system. The concentration of magnesium chloride, deoxynucleoside triphosphates, Taq DNA polymerase, and temperature-cycling profiles can all affect the specificity and, consequently, the efficiency of the amplifications. For HIV, we have designed primers that are specific for either HIV-1 or -2 as well

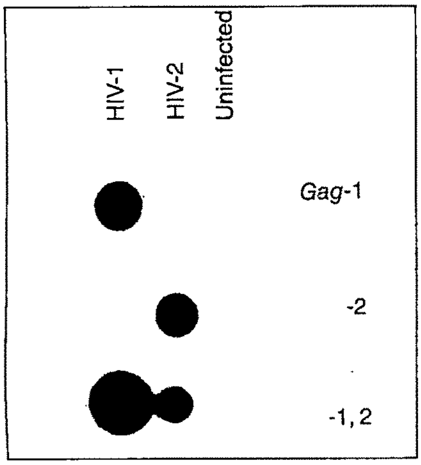

Fig 2.—Dot blot analysis of DNA extracted from individuals infected with human immunodeficiency virus (HIV) 1 or 2 that were amplified either with primers that are specific for both HIV-1 and -2 or with primers that are specific for each virus.

as primers that detect both viruses (Fig 2). For specific amplification of either HIV-1 or -2, regions with the least homology between the two viruses were targeted. On the other hand, only regions conserved between the two viruses were selected for simultaneous amplification.

Although the presence of large numbers (ie, 10 000) of HIV molecules in the original sample will result in amplification products that can be visualized on agarose gels following ethidium bromide staining, often the numbers of copies of HIV proviral DNA in a sample are small and may be as few as one copy in 10 000 cells.[11] The presence of small numbers of HIV molecules in a background of highly complex genomic DNA often results in the amplification of nonspecific products. Because the nonspecific products may be of the same length as the expected fragment, confirmation of the correct product is necessary and can be achieved through the use of a labeled probe that is complementary and between the primers of one strand of the amplified product. In addition to providing an extra level of specificity, the use of phosphorus 32–labeled probes provides the sensitivity often required for detection of products of low-copy number samples. When PCR is coupled with a detection method known as oligomer hybridization,[26] as few as 18 molecules of HIV in a background of 150 000 peripheral blood mononuclear cells or 1 μg of DNA can be detected.[27] Although purified DNAs were initially used, crude DNAs prepared by lysis of whole cells with proteinase K and nonionic detergents are now being used (Higuchi R., unpublished data, 1988, and reference 26). The use of Ficoll-Hypaque fractionated cells provides for the detection of HIV in not only CD4$^+$ cells, but in all mononuclear cells.

When PCR is used for detection of low copy targets such as HIV, extreme care must be taken to avoid cross-contamination of samples or carry over of amplified products that can lead to false-positive results. Guidelines for avoiding false-positive results with PCR have been described.[28,29] These guidelines include the using of positive displacement pipettes, the preparing and aliquot partitioning of reagents in an area that is free of PCR-amplified products, the sterilizing of reagents, and the frequent changing of gloves. Careful choice of positive and negative controls is important. Specifically, several "no DNA" reagent controls and negative sample controls should be included with each set of amplifications. The positive control should have a small number of target molecules so as to avoid the unnecessary generation of a large number of amplified targets. In addition, a small number of target molecules will also enable a better assessment of the efficiency of an amplification. A strict adherence to these guidelines has resulted in a dramatic reduction in the frequency and amount of carry over.

Polymerase chain reaction has been used for the direct detection of HIV-1 proviral DNA sequences in peripheral blood mononuclear cells of infected individuals, and has demonstrated clinical utility in several areas of diagnostics. For example, because of the window between infection and seroconversion, antibodies may not be detected in an infected individual. Although this seronegative window is typically 6 weeks to 6 months, protracted periods without production of antibodies have been described.[30,31] The PCR procedure detected the presence of HIV in high-risk individuals at least 6 months prior to seroconversion. A small number of individuals, initially identified as culture positive, demonstrated signs of infection by PCR for as long as 24 to 39 months prior to seroconversion. The observations noted here merit replication and further analysis.[3]

In seropositive individuals, PCR has been used to confirm the presence of HIV infection in individuals who were virus culture and/or antigen[25,32] negative. This procedure is currently being used in reference laboratories where HIV-1 proviral sequences have been detected in greater than 97% of the antibody positive individuals (Pathology Institute, Berkeley, Calif, and Specialty Laboratories, Santa Monica, Calif, unpublished data, 1989).

The procedure has begun to contribute to the resolution of the infection status of individuals with indeterminate serologic findings. In a small study of 100 low-risk volunteer blood donors with Western blot indeterminate patterns, Jackson et al[33] found that no one harbored HIV proviral sequences.

Polymerase chain reaction has been successfully used as a prognostic marker for infected infants born to seropositive mothers.[34-36] The persistence of maternal antibodies in babies for up to 18 months makes diagnosis by serologic assays difficult. When PCR is used to screen babies, those who were positive by PCR were either clinically ill or later showed signs of infection. Infants negative by PCR were later found to be seronegative and have remained clinically healthy. While the number of infants studied is small, the data thus far suggest that PCR can play an important role in the early identification of infected infants.

Polymerase chain reaction was used to confirm the first case of HIV-2 in a West African undergoing treatment in the United States.[37] In addition, the procedure was used to confirm the first documented case of a West African who was coinfected with HIV-1 and -2.[38] Similarly, individuals who were coinfected with HIV and human T-cell lymphotropic virus I were identified by PCR.[39,40]

Polymerase chain reaction has proved to be a valuable tool in several areas of research. First, the procedure has been used to study genetic variability in HIV-1 isolates.[22,33] Conventional means of restriction fragment polymorphism, although informative, provides only a limited assessment of heterogeneity. With PCR, the population of viruses, both in vivo and in vitro, can be analyzed. The sequences obtained from such studies should reflect more accurately the virus sequences present in an individual, as it does not require cultivation that may result in strain selection. Second, the demonstration that DNA extracted from formalin-fixed, paraffin-embedded tissue can serve as a template for PCR[41] suggests that archival material can be used to further elucidate the epidemiology of the virus. Third, experiments on the quantitation of PCR product suggest that the procedure could be used for the monitoring of viral load in patients receiving therapeutic agents.[42]

In summary, PCR has played an important role in (1) detecting of HIV sequences in early infection (2) identifying HIV sequences in seropositive individuals whose cells were culture negative for HIV after cocultivation and/or whose serum samples were negative for viral antigens, (3) prognostic screening of babies born to seropositive mothers, (4) resolving the infection status of individuals with indeterminate Western blot patterns, and (5) the typing of the human retroviruses present. The development of more rapid and inexpensive colorimetric assays will make the test more amenable for large-scale screening. In addition, PCR is expected to contribute to the diagnosis and study of other retroviruses.[29,43]

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Centers for Disease Control Perspective on Quality Assurance for Human Immunodeficiency Virus Type 1 Antibody Testing

Model Performance Evaluation Program

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• Ensuring high quality in human immunodeficiency virus type 1 (HIV-1) antibody testing is an essential component of the organized public health response to epidemic HIV-1 infection. In 1986, the Centers for Disease Control designed the Model Performance Evaluation Program to assess and improve the analytic quality of HIV-1 antibody testing. In addition, the program was designed to gather information about HIV-1 antibody testing practices. The utility of this information is in identifying potential barriers to quality throughout the total testing process. Currently, 1405 laboratories participate in the program. Participating laboratories are located both within and outside the United States and consist primarily of hospitals, blood banks, health departments, and independent laboratories. The responses to a questionnaire completed by 1050 program-participant laboratories in September 1988 suggest that at several stages in the HIV-1 antibody total testing process, laboratory practices (including the interpretation of Western blot patterns) are variable and that standardization of these practices would improve quality.

(*Arch Pathol Lab Med.* 1990; 114:263-267)

The Centers for Disease Control's (CDC's) Public Health Practice Program Office (PHPPO), formerly known as the Training and Laboratory Program Office, has a long history of promoting quality in laboratory testing. Between 1969 and 1986, PHPPO conducted a comprehensive proficiency testing program and inspected interstate laboratories under the provisions of the Clinical Laboratory Improvement Act of 1967. Although PHPPO's proficiency testing activities have since been assumed by a variety of professional and commercial organizations, such as the College of American Pathologists and the American Association of Bioanalysts, PHPPO remains vitally interested in the role that laboratory quality plays in ensuring health outcomes of high quality. The philosophy of PHPPO's approach to laboratory quality assurance is exemplified by our activities in human immunodeficiency virus type 1 (HIV-1) antibody testing performance evaluation.

A proficiency testing program for laboratories performing HIV-1 antibody testing was instituted by the CDC in 1985, shortly after the first enzyme immunoassay for detecting HIV-1 antibody was commercially licensed.[1] The first survey contained 10 samples (five pairs of duplicates), although subsequent surveys were reduced to 6 samples (usually three duplicate pairs). Samples were presented as duplicates so that within- and between-survey reproducibility could be measured. This program was designed so that laboratories performing HIV antibody testing could measure their analytic performance "under actual testing conditions."[1] Because the samples were purposely created to measure technical competence, they included a higher percentage of samples with intermediate and low reactivity than might be encountered in the usual clinical situation.

Interest in this program was great, and by 1986 the number of participating laboratories had increased from 50 to 475.[1] But in 1987, PHPPO implemented substantial programmatic changes in its approach to HIV-1 proficiency testing. In addition to ensuring the analytic quality of testing, the Model Performance Evaluation Program (MPEP) was designed to gather information about the preanalytic and postanalytic stages in the HIV-1 antibody testing process through the use of a survey and a prospective study of HIV-1 reporting practices. The latter is being conducted through a cooperative agreement with San Diego (Calif) State University, Graduate School of Public Health.

Concern about the influence of preanalytic and postanalytic actions or events on testing quality grew out of the 1986 Institute on Critical Issues in

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Health Laboratory Practice.[2] One of the recurrent messages of that gathering, long known to many laboratorians, was the caveat that testing quality is a global concept, encompassing every step from preparing the patient and collecting the specimen, through testing itself, to reporting the result and making the subsequent clinical decision, which is based on the test result.

Table 1.—Comparison of US/US Territorial and Non-US Laboratories Enrolled in the Model Performance Evaluation Program, by Purpose of HIV-1 Antibody Testing*

	No. (%) of Laboratories	
Purpose for Testing	**US/US Territorial (n = 995)**	**Non-US (n = 55)**
Diagnostic testing	765 (77)	44 (80)
Screening of blood for transfusion	384 (39)	19 (34)
Anonymous screening	190 (19)	13 (24)
Basic research	99 (10)	15 (27)
Clinical testing for anti-HIV drug trials	48 (5)	5 (9)

*HIV-1 indicates human immunodeficiency virus type 1. Testing purpose categories are not mutually exclusive.

DESCRIPTION OF THE MPEP

Enrollment in the MPEP is voluntary and free of charge, and participants are identified through letters to laboratories, manufacturers, and professional organizations as well as by word of mouth. All participants perform HIV-1 antibody testing, though the number and types of HIV-1 antibody tests they offer vary. Currently, 1405 laboratories are enrolled in the MPEP. Most are located in the United States, although laboratories in three US territories and 82 other countries also participate in this program. Each of the 50 states has laboratories enrolled in the MPEP, with California, Texas, Illinois, Florida, Pennsylvania, and New York accounting for approximately 41% of all US participants.

To evaluate the analytic quality of testing and to assist laboratories in self-improvement we mail panels of samples for HIV-1 antibody testing to MPEP participants. The participants are asked to treat these samples in the same manner they would patient specimens. The first three panels each contained 22 samples (11 pairs of blind replicates), which ranged from nonreactive through weakly reactive to strongly reactive. To date, each survey panel has contained both single-donor and pooled-donor samples. The samples, collected and prepared for CDC by an outside contractor, are obtained from blood collection centers. To locate weakly reactive samples from early HIV-1 seroconverters, CDC's contractor is currently attempting to locate anonymous donors through clinics for the treatment of sexually transmitted diseases. Before they are used in the panel, all samples are tested at CDC and by the contractor. The sample is validated by 36 reference laboratories, which also participate in the actual program. Within each shipment, panel configuration varies, and not all laboratories receive the same samples.

Participants report their results by mail to the CDC on a specially designed form and subsequently receive three reports from the MPEP. The first report includes the results of the reference laboratories, grouped by test method and kit. The second is a summary tabulation of all participants, and the third is an analysis in which results are grouped for each evaluation sample, by test kit manufacturer, test method, and Western blot (WB) band pattern interpretation. All participants are identified by a unique, confidential code number, which is used to report their results to the CDC.

The process of testing the panels provides both the participant and the CDC with useful information. However, this information largely reflects the analytic testing aspects of HIV-1 antibody determination. To learn more about the other variables associated with HIV-1 testing quality, the MPEP performs a separate biannual survey of participants. The remainder of this article reports the results of the September 1988 participant survey.

DESCRIPTION OF PARTICIPANTS

In the MPEP, 1381 participants were surveyed and 1050 responded—995 from the United States or one of its territories and 55 from other countries. United States and territorial respondents have been further subclassified into one of four self-identified laboratory categories: hospital (39% of US and territorial respondents); health department (8%); blood bank (13%); or 'other" (39%). The other category includes independent laboratories (52%); employee health clinics (1%); clinics for the treatment of sexually transmitted diseases (2%); drug screening laboratories (2%); physician-office laboratories (1%); HIV test manufacturers (4%); and laboratories that classified themselves in multiple categories (36%).

Table 1 summarizes the self-reported purposes for HIV-1 antibody testing, comparing US and territorial participants with those from other countries. Not surprisingly, diagnostic testing and screening of blood before transfusion were the two most frequently cited reasons among all participants.

SURVEY RESULTS

When laboratories were asked to estimate the number of specimens they had tested for HIV-1 antibody since testing began, blood bank laboratories reported the highest volume, with nearly 60% responding that they had tested between 100 000 and 1 million specimens. Health department laboratories were next in frequency, with 56% reporting a cumulative volume between 5000 and 50 000. Forty percent of laboratories falling into the "other" category reported testing between 1000 and 10 000 specimens, and 62% of hospital laboratories had tested between 100 and 5000 specimens.

Table 2 gives the source of "outside" specimens (ie, those not collected within the institution providing care) for each of the US and territorial laboratories surveyed. The categories of specimen source are not mutually exclusive and demonstrate that, regardless of site, a majority of laboratories performing HIV-1 testing receive specimens from a variety of clinical settings. This implies that specimens are from heterogeneous populations or groups; some of those tested are probably at great risk for HIV-1 infection, while others are at much lower risk of being infected. The large number of outside sources for specimens also implies that many laboratories may have

Table 2.—Source of 'Outside' Specimens to Be Tested for HIV-1 Antibody*

	Type of Laboratory			
Source of Specimens	Hospital (n = 391)	Health Department (n = 84)	Blood Bank (n = 128)	Other (n = 392)
Hospital	182 (46)	59 (70)	93 (73)	240 (61)
Sexually transmitted disease clinic	47 (12)	68 (81)	11 (9)	98 (25)
Family planning center	36 (9)	52 (62)	12 (9)	82 (21)
Drug abuse treatment center	39 (10)	49 (58)	6 (5)	105 (27)
Alternate testing site	37 (9)	68 (81)	16 (12)	69 (18)
Private physician	206 (53)	52 (62)	68 (53)	265 (68)
Health maintenance organization	39 (10)	26 (31)	13 (10)	94 (24)
Out of state	23 (6)	5 (6)	19 (15)	90 (23)
Other	112 (29)	39 (46)	53 (41)	125 (32)

*HIV-1 indicates human immunodeficiency virus type 1. Values are number (percent). Sources of specimens may not be mutually exclusive. All laboratories are United States and territorial.

Table 3.—Laboratory Personnel Training Requirements Before Performing HIV-1 Antibody Testing*

	Type of Laboratory			
Type of Training	Hospital (n = 391)	Health Department (n = 84)	Blood Bank (n = 128)	Other (n = 392)
In-house	390 (99)	79 (94)	128 (100)	385 (98)
State Health Department	5 (1)	23 (27)	0 (0)	9 (2)
CDC workshop	7 (2)	9 (11)	0 (0)	6 (1)
Manufacturer's workshop	101 (26)	30 (36)	25 (19)	123 (31)
Reading kit instructions	213 (54)	44 (52)	81 (63)	216 (55)
Other	17 (4)	7 (8)	17 (13)	18 (5)
None	0 (0)	0 (0)	0 (0)	2 (<1)

*HIV-1 indicates human immunodeficiency virus type 1; CDC, Centers for Disease Control. Values are number (percent). Training categories are not mutually exclusive. All laboratories are in the United States and US territories.

Table 4.—Sequence of HIV-1 Antibody Tests Performed In-house*

	Type of Laboratory			
Testing Algorithm	Hospital (n = 68)	Health Department (n = 61)	Blood Bank (n = 20)	Other (n = 105)
SDW	25 (37)	17 (28)	12 (60)	37 (35)
DDW	5 (7)	0 (0)	0 (0)	4 (4)
SDI	3 (4)	8 (13)	0 (0)	2 (2)
SSW	8 (12)	4 (6)	0 (0)	10 (9)
DW	3 (4)	1 (2)	0 (0)	4 (4)
SDWI	3 (4)	7 (11)	2 (10)	4 (4)
SSI	0 (0)	3 (5)	0 (0)	1 (<1)
SW	3 (4)	0 (0)	0 (0)	2 (2)
Other	18 (26)	21 (34)	6 (30)	41 (39)

*Includes only those US and US territorial laboratories performing confirmatory tests on-site. HIV-1 indicates human immunodeficiency virus type 1; SDW, single enzyme immunoassay (EIA) followed by duplicate EIA followed by Western blot (WB); DDW, duplicate EIA followed by duplicate EIA followed by WB; SDI, single EIA followed by duplicate EIA followed by indirect immunofluorescence (IIF); SSW, single EIA followed by single EIA followed by WB; DW, duplicate EIA followed by WB; SDWI, single EIA followed by duplicate EIA followed by WB followed by IIF; SSI, single EIA followed by single EIA followed by IIF; SW, single EIA followed by WB; and other, algorithms not described. Values are number (percent).

little or no direct control over specimen labeling, collection, and transportation procedures.

Participants' responses to a question about the work shift during which HIV-1 testing was performed show that most respondents (except the blood bank laboratories) consider HIV-1 testing a routine procedure and usually perform it only during the day shift. Understandably, blood banks were more likely to report testing for HIV-1 antibody during evenings, nights, weekends, and holidays. Regardless of site, most laboratories perform enzyme immunoassay (EIA) screening for HIV-1 antibody more frequently than once a week. A majority of laboratories in each category reported testing for HIV-1 antibody 5 or more days each week.

In response to a question about the kind of training that laboratory personnel must complete before performing HIV-1 antibody testing, most participants identified the following three resources: in-house training, manufacturer's kit instructions, and manufacturer's workshops (Table 3).

Fifty-four (98%) of the 55 non-US participants and 991 (99%) of the 995 US and territorial participants reported that they performed HIV-1 antibody determination by EIA. A much smaller proportion of all participants reported that they performed WB confirmatory testing in-house: 67% of non-US laboratories and 34% of all US and territorial laboratories. As a group, health department laboratories are more likely to perform their own WB analysis than are other types of laboratories. When asked whether they performed indirect immunofluorescence assays for HIV-1 antibody in-house, 62% of the non-US respondents and 34% of the US and territorial respondents answered that they did. Sixty-nine percent of the surveyed non-US laboratories and 82% of the surveyed US and territorial laboratories indicated that they send HIV-1 specimens to other laboratories for supplemental testing. Most participants identified WB testing as the most frequently requested supplemental test.

Laboratories were asked to describe the sequence of testing events (for reactive specimens) for HIV-1 antibody tests that they perform on-site. Table 4 summarizes testing algorithms only for those US laboratories performing confirmatory tests in-house. The single most common pattern to emerge was testing by single EIA followed by duplicate EIA followed by WB. Although large, the "other" category of testing algorithms comprises a multiplicity of patterns.

Responses to a question about which WB band patterns were interpreted to be HIV-1 antibody positive (reactive) were aggregated for all US and territorial and non-US participants who performed the test in-house. The aggregated results showed that the largest proportion (30%) considered the following pattern indicative of infection: p24/25, plus p31/32, and either gp41 or gp120/gp160 (Food and Drug Administration–licensed DuPont test criteria). The next most frequently identified pattern was "any two of p24/25, gp41, gp120-gp160" (Association of State and Territorial Public Health Laboratory Directors, CDC, and Department of Defense criteria), with 24% agreement. Fifteen percent indicated that "p24/25 plus gp41" (National Institutes of Health) should be interpreted as positive (reactive), 12% endorsed one protein from each of the three viral gene product groups as positive (American Red Cross criteria), 10% identified proteins from two of the three viral gene product groups as positive (Consortium for Retrovirus Serology Standardization criteria), and the remainder indicated that they used other criteria in the interpretation of a positive WB result. None of the four categories of US and territorial laboratories (ie, hospital, health department, blood bank other) reported just one criterion for classifying WB results as positive. When responses to the question about what constitutes a negative WB interpretation were aggregated, 54% of all respondents indicated "no bands present" and 40% indicated "no HIV-specific protein bands present." Six percent indicated other criteria for the interpretation of negative WB tests.

A variety of questions pertaining to HIV-1 antibody testing practices are given in Table 5. As seen in that table, a sizable minority of laboratories do not have written criteria for determining when specimens for HIV-1 antibody testing are unsatisfactory. Most laboratories surveyed did have written procedures for protecting the confidentiality of HIV-1 test results. Of interest is the fact that certain US and territorial laboratories in each of the four categories continue to treat specimens with heat inactivation, presumably as a safety practice, and that only a minority of hospital and blood bank laboratories use control serum samples other than those supplied by the kit manufacturer

Table 5.—HIV-1 Antibody Testing Practices by Laboratory Type*

	No. (%) Responding Yes by Type of Laboratory			
Survey Items	Hospital (n = 391)	Health Department (n = 84)	Blood Bank (n = 128)	Other (n = 392)
Does your laboratory have written criteria for unsatisfactory HIV specimens?	346 (88)	64 (76)	94 (73)	324 (83)
Does your laboratory pretreat specimens by heat inactivation?	7 (2)	13 (15)	1 (<1)	11 (3)
Does your laboratory pretreat specimens by addition of detergent?	7 (2)	0 (0)	0 (0)	11 (3)
Do you routinely use control sera other than those supplied by the manufacturer?	64 (16)	51 (61)	16 (12)	90 (23)
Do you send HIV specimens to other laboratories for supplemental testing?	342 (87)	49 (58)	113 (88)	310 (79)
Do you have procedures for protecting the confidentiality of HIV test results?	372 (95)	84 (100)	127 (99)	376 (96)

*HIV-1 indicates human immunodeficiency virus type 1. Values are number (percent). All laboratories are in the United States and US territories.

Table 6.—HIV-1 Antibody Testing by Enzyme Immunoassay: Number of Controls Used, by Laboratory Type*

	Type of Laboratory			
	Hospital	Health Department	Blood Bank	Other
		Positive Controls per Run		
	(n = 364)	(n = 82)	(n = 118)	(n = 365)
0	0 (0)	0 (0)	0 (0)	0 (0)
1	40 (11)	7 (8)	8 (7)	43 (12)
2	51 (14)	12 (15)	15 (13)	88 (24)
3	220 (60)	37 (45)	72 (61)	183 (50)
≥4	53 (15)	26 (32)	23 (19)	51 (14)
		Negative Controls per Run		
	(n = 363)	(n = 80)	(n = 118)	(n = 364)
0	1 (<1)	0 (0)	0 (0)	0 (0)
1	11 (3)	2 (2)	1 (<1)	23 (6)
2	241 (66)	47 (59)	92 (78)	201 (55)
3	93 (26)	26 (32)	22 (19)	120 (33)
≥4	17 (5)	5 (6)	3 (2)	20 (5)

*Excludes laboratories that failed to respond to the question. HIV-1 indicates human immunodeficiency virus type 1. All laboratories are in the United States and US territories. Values are number (percent).

Table 7.—Estimated Percentage of Specimens Reactive for HIV-1 Antibody by Enzyme Immunoassay, Within Most Recent Week of Survey, by Laboratory*

	Type of Laboratory			
Estimated % Reactive	Hospital	Health Department	Blood Bank	Other
<1	53	16	89	55
1-5	23	59	9	26
6-10	9	16	<1	9
11-49	13	6	2	7
50-100	2	2	0	3

*HIV-1 indicates human immunodeficiency virus type 1. All laboratories are in the US and US territories.

Table 6 describes the number of positive and negative controls used when testing for HIV-1 antibody by EIA, and Table 7 summarizes the participants' estimates of the proportion of reactive samples (HIV-1 antibody testing by EIA), "during the most recent representative week."

COMMENT

Ensuring the high quality of HIV-1 antibody testing is an essential component of the organized public health response to epidemic HIV-1 infection. Accurate and timely HIV-1 testing plays a critical role in programs for disease surveillance and prevention and continues to ensure the availability of a safe national blood supply. From a medical perspective, critical treatment decisions are based on the results of HIV-1 antibody testing.

Although great technical strides have been made in the field of HIV-1 diagnostics and although testing for HIV-1 antibody has become widely available, it is important to emphasize that quality assurance for HIV-1 testing is an ongoing activity and not merely a function of the novelty of the diagnostic procedure. As public health messages encourage more and more people to receive counseling and testing, we can expect a proliferation of HIV-1 testing in response to this need. The important public health, medical, and social outcomes associated with HIV-1 antibody testing make it imperative that testing be of the highest quality possible, within the limits of the technology.

Nor is it only the analytic portion of the testing process that warrants quality assurance. Early in 1987, a survey of American hospitals performing HIV-1 antibody testing showed "marked variety" in the manner in which results were handled, patient consent was obtained, and counseling was performed.[3] The components of laboratory testing quality encompass the entire testing process from test requisition and specimen collection through result reporting and interpretation.[4] The CDC's MPEP approaches the goal of laboratory quality assurance comprehensively, recognizing that preanalytic and postanalytic variables can have a major effect on the quality of test results and their subsequent clinical interpretation.

Much of the data collected by the MPEP can be used to identify potential barriers to quality throughout the testing process. One of the most immediate needs identified in this survey pertains to a postanalytic component of HIV-1 antibody testing, namely, the interpretation of WB results. Our survey showed that laboratories, regardless of type, use a variety of criteria for classifying WB results as positive or negative, and we suspect that even within the same laboratory different criteria may be used under different circumstances. For clinical and public health practice, the CDC recommendations, which endorse the Association of State and Territorial Public Health Laboratory director's guidelines for a positive WB as the presence of any two of p24, gp41, and gp120/160 bands,[5] should help to rectify this situation. Ongoing educational efforts, however, will still be required to disseminate this information to laboratories performing the WB procedure and to clinicians ordering the test.

Our observations that not all laboratories use the same algorithm for performing HIV-1 testing and that the number of controls used during EIA testing for HIV-1 is variable suggest the need for standardization, and for ongoing education and training in the analytic stages of HIV-1 antibody testing.

Examples of potential barriers to quality in the preanalytic stage of testing are the lack of written criteria for the rejection of unsatisfactory HIV-1 specimens and the practice, albeit uncommon, of heat inactivating specimens before testing. This practice is unnecessary as a safety precaution for clinical specimens if laboratories have instituted universal precautions for all blood and body fluid specimens.[6] In addition, heat inactivation has been shown to interfere with test accuracy both for EIA screening procedures[7,8] and for WB confirmatory testing.[9] Furthermore, the large number of participants reporting receipt of outside specimens suggests another potential source of preanalytic error in specimen collection, labeling, and transport.

Although overwhelming concern about the need for quality HIV-1 testing often forces the public sector to look for deficits in the testing process, it is encouraging that the MPEP has documented a number of practices in the preanalytic, analytic, and postanalytic stages of HIV-1 antibody testing that indicate that a large number of laboratories are providing high-quality testing. Furthermore, the fact that the CDC program is voluntary and yet manages to attract a large number of participants indicates that many laboratorians share our concern about the importance of high quality in HIV-1 testing.

The information obtained by the MPEP is germane to other public health programs as well. Certain elements of the data we collect will be used to develop a more comprehensive understanding of the training needs of laboratorians performing HIV-1 antibody testing and can be incorporated into laboratory training interventions that seek to promote standards of quality across different testing sites. Furthermore, our experience with the MPEP can be applied to other laboratory diagnostic procedures. The CDC's emphasis on ensuring high quality of HIV-1 antibody testing is an expression of our recognition of the important role that laboratory testing plays in supporting national public health objectives.

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Human Immunodeficiency Virus Type 1 Proficiency Testing

The American Association of Blood Banks/College of American Pathologists Program

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• The American Association of Blood Banks/College of American Pathologists Viral Marker Survey program added samples to evaluate participant performance with test systems for the detection of antibodies to human immunodeficiency virus type 1 in 1985. On the 80 challenges sent through April 1989, the major problem observed with enzyme immunoassay testing was unexpected reactivity on samples that did not contain anti–human immunodeficiency virus type 1. One manufacturer's testing system accounted for most of the specificity problems. The sensitivity of the enzyme immunoassay for anti–human immunodeficiency virus type 1 is close to 99%, based on the multiple reactive samples used on the 16 panels. Between 1985 and 1989, there was a 14-fold increase in participants reporting Western blot results. The introduction of a licensed system for Western blots increased the number of samples that contained antibody to human immunodeficiency virus type 1 interpreted as indeterminate. Answers to supplementary questions showed that the rate of repeatably reactive and confirmed positives on blood donors dropped to less than 0.1%.

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On March 2, 1985, reagents to screen blood donors for anti–human T-cell lymphotropic virus type III (now called anti–human immunodeficiency virus type 1 [HIV-1]) were licensed by the Food and Drug Administration to assure a safer blood supply. The initial kits were based on enzyme immunoassay (EIA) systems that used a stock National Institutes of Health viral culture provided to five manufacturers under an RFP. In anticipation of the availability of these test kits, the College of American Pathologists (CAP) Transfusion Transmitted Viruses Ancillary Committee began planning for a pilot survey to evaluate the use of these new tests in the routine screening setting. In July 1985, the first of three pilot surveys was mailed. In 1986, samples for anti–HIV-1 testing were incorporated into the Donor Module of the American Association of Blood Banks (AABB)/CAP Viral Markers Survey (W) for quarterly distribution to subscribers. As with the pilot surveys, each mailing has had five challenges to be evaluated by routine screening and confirmation tests. In 1989, a new survey (WZ) was introduced for laboratories doing Western blotting.

In planning for evaluating laboratory performance with anti-HIV test methods, the committee developed specifications to include reactive and nonreactive samples that should be correctly identified by using the same tests and methods used in testing blood donors. As described below, replicate samples within mailings and between mailings are used to monitor the reproducibility of results and consistency of reagent kits. To help recognize problems with these tests, supplementary questions about methods, reagents, and observed results on unknown samples are included with each mailing. These questions are also designed to determine the common approaches to various policies on issues, such as reporting, confidentiality, confirmation of results, etc.

MATERIALS AND METHODS

Participants in the AABB/CAP Viral Markers (Donor Module) Survey are sent

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five challenges each quarter. The samples are made from pools of blood donor plasma that have been defibrinated by the addition of thrombin and calcium chloride, delipidized, and sterile filtered. Thimerosal (0.01%) is used as a preservative. Units used to prepare the samples are tested with a Food and Drug Administration–approved anti-HIV EIA reagent that is normally used for screening of donors. Blood donor units found to be HIV reactive by both EIA and a Western blot are added to pools of nonreactive plasma in varying dilutions. The dilution used is selected to react repeatably with currently available lots of screening reagents and to show expected viral bands on Western blot. All reactive units are heated to 56°C for 45 minutes to reduce potential infectivity.[2-4] Nonreactive units are not heat-treated. Because of concerns about infectivity, the reactive samples used on the pilot surveys were prepared from dilutions of immunoglobulin preparations for human use that contained high titers of anti-HIV.

Results obtained by subscribers to the Viral Marker Survey are returned to the CAP Computer Center in Traverse City, Mich, for tabulation and evaluation. Participant results are compared with results obtained by referees by using similar methods. If less than 80% of participants agree on the result, a sample is considered to be a nonconsensus. Grading of results is based on the consensus of the reports for each method, not on the manufacturer of the reagents or kit used. Results from EIA screening are compared by reagent manufacturer. This comparison is based on the total number of correct responses reported for all reactive or all nonreactive samples divided by the total number of expected responses. Participants who use Western blotting are asked to report both the bands observed and the interpretation of the test. Photocopies of the membranes are also requested. Starting with panels in the 1988 survey year, results on Western blot also have been compared by reagent manufacturer.

RESULTS

This report summarizes the results from 16 mailings between July 1985 and April 1989. The total number of samples sent was 80 and included 46 reactive samples and 34 nonreactive samples. Table 1 compares, by method, the number of participants reporting results for each survey year. From the initiation of the survey in 1985 to the present time, there has been a threefold increase in laboratories doing EIA screening and a 14-fold increase in those reporting Western blot results.

Table 2 shows the results reported by method for the 80 samples. Participants who used EIA screening correctly identified the 46 reactive samples 98.58% of the time. Almost all of the incorrect responses could be attributed to one replicated sample (W-26 and W-41) used in 1988 (Table 3). On both mailings, including this sample, 20% to 30% of participants who used three of the six EIA reagents on the market reported the samples as nonreactive. The majority of participants who used these three reagents and 97.1% to 99.6% of those who used reagents from the other manufacturers reported the samples as reactive.

Consensus results (80% participant agreement) by EIA were obtained on 25 (73.5%) of the 34 nonreactive samples. Table 3 shows that almost all of the reactive results on these samples were reported by participants who used reagents from a single manufacturer. When the same sample was replicated within a mailing (WA-1 [1987] and WA-5 [1987]), there was a concordance in the number of participants reporting both samples as reactive. When results from between panel replicates (WM-2 [1985] and WM-9 [1985]) were compared, reactive rates on the same sample differed by as much as 72%. The incorrect responses correlated with the lot of reagent used by participants.

Results obtained by participants who used Western blotting are shown in Table 4. On the first nine panels (pilot surveys and WA [1986] and WB [1987]), participants' results were summarized as reactive (including indeterminate results) or nonreactive. Starting with panel WC (1987), indeterminate results were shown in the summaries. Results on the first nine panels showed that

Table 1.—Methods Used for HIV-1 Antibody Testing by Subscribers to the AABB/CAP Viral Marker Survey*

	No. of Laboratories Reporting		
Year	EIA	WB	IFA
1985	445	11	0
1986	453	39	0
1987	698	83	16
1988	899	153	24
1989	982	141	22

*HIV-1 indicates human immunodeficiency virus type 1; AABB, American Association of Blood Banks; CAP, College of American Pathologists; EIA, enzyme immunoassay; WB, Western blot; and IFA, immunofluorescence assay.

Table 2.—Percentage of Correct Results Reported by Participants Using EIA Tests for Antibody to HIV-1*

	Expected Result	
Year	Nonreactive†	Reactive
1985	90.6 (6)‡	98.9 (9)‡
1986	98.7 (8)	98.1 (12)
1987	60.3 (9)	99.6 (11)
1988	97.9 (7)	97.2 (13)
1989	79.0 (3)	99.5 (2)

*EIA indicates enzyme immunoassay; HIV-1, human immunodeficiency virus type 1.

†Nine nonconsensus samples (1985 two; 1986, one; 1987, five; and 1989, one).

‡Number of samples on panels is given in parentheses throughout.

Table 3.—Comparison of Results by Manufacturer on Selected Samples*

		% Agreement		
		EIA		
Sample ID No. (Year)	Correct Result	Abbott Laboratories, North Chicago, Ill	Others†	WB
W-2 (1985)	Nonreactive	19.7 (289)‡	100 (30)	72.2 (11)
W-9 (1985)	Nonreactive	93.1 (299)	100 (46)	90.0 (10)
W-35 (1986)	Nonreactive	2.0 (350)	93.6 (130)	97.1 (38)
W-1 (1987)	Nonreactive	26.5 (350)	99.1 (60)	90.9 (33)
W-5 (1987)	Nonreactive	27.0 (353)	99.0 (60)	96.0 (33)
W-22 (1987)	Nonreactive	64.8 (495)	100 (177)	93.0 (83)
W-5 (1989)	Nonreactive	30.7 (721)	96.7 (338)	98.6 (141)
W-26 (1988)	Reactive	99.9 (627)	73.7 (164)§	97.8 (148)
W-41 (1988)	Reactive	99.8 (648)	76.9 (173)§	96.4 (157)

*ID indicates identification; EIA, enzyme immunoassay; and WB, Western blot.

†Dupont Pharmaceuticals, Wilmington, Del; ElectroNucleonics; Genetic Systems Corp, Seattle, Wash; Organon Teknika, West Orange, NJ; Ortho, Raritan, NJ.

‡Number of reporting participants is given in parentheses throughout.

§Participants using Dupont, ElectroNucleonics, and Ortho reagents.

97.4% of the time participants reported the 27 reactive samples correctly. Correct responses were given 94.9% of the time on the 18 nonreactive samples. Seven panels were mailed from October 1987 through April 1989. The percentage of correct responses for the 20 reactive samples on these panels dropped to 82.4%.

Table 5 shows Western blot results by reagent source. Between 0.8% and 1.4% of participants reported reactive samples as nonreactive; however, almost 30% of laboratories that used licensed reagents interpreted these samples as indeterminate. Few participants reported the 15 nonreactive samples as reactive. The percentage of participants who reported these samples as indeterminate was between 1.6% and 12.5%, depending on the reagent system used.

Supplementary questionnaires that were sent with the survey samples requested information on the technical performance of the tests, the numbers of patients who were described to have developed acquired immunodeficiency syndrome as a result of transfusion, and participant policies on various acquired immunodeficiency syndrome–related issues. Table 6 shows that from 1985 to the present there has been a significant decrease in the number of donors with initial reactive and repeatably reactive results on routine EIA testing. There also has been a fourfold decrease in the number of donors confirmed as anti-HIV reactive.

COMMENT

The licensure of screening tests for antibody to HIV-1 was accompanied by announcements that this test was specifically designed to protect the safety of the blood supply. The package inserts for these test systems clearly specified that the approved use was for testing normal blood donors. Prelicense testing of the reagents indicated nearly 100% sensitivity when panels of samples from patients with acquired immunodeficiency syndrome were evaluated. Specificity was also reported to be greater than 99% on samples from normal individuals. Because there is a possibility that reactivity on the screening test could be due to technique-dependent variables, protocols for determining reactivity require duplicate evaluation of initially reactive samples. As seen in Table 6, the percentage of initial reactive samples observed in blood donors has decreased with time. This decrease in reactivity can be attributed to several factors, including more experience with anti-HIV testing, modifications made in the reagent systems, and a change in the population being tested.

Much concern has been expressed that the application of these tests to large numbers of persons in populations with a low prevalence of HIV-1 would lead to a high proportion of false-positive to true-positive results.[5-8] Results that have reported on testing large numbers of donors have confirmed that only a small number of initially reactive samples are from individuals with antibody to HIV-1.[9,10] The significance of the specificity of HIV-1 antibody tests has led to much debate, as various proposals were advanced for the screening of additional populations, ie, premarital applicants,[11] military recruits,[12] immigrants, etc.

Results that have reported on the AABB/CAP Viral Marker Survey specimens have shown that with the samples used, specificity is a problem.[13] Of the 80 challenges reviewed in this article, 9 (27%) of 33 nonreactive samples were classified as a nonconsensus because more than 20% of participants reported their result as repeatably reactive by EIA (Table 2). As seen in Table 3, almost all of the nonspecificity could be assigned to participants who used reagents that were made by Abbott Laboratories, North Chicago, Ill. Extensive studies of donor units selected for pooling to obtain survey samples, as well as on the final pools at various stages in processing, have failed to delineate why this specificity problem occurs. The variability in results obtained on the same survey sample with different lots of Abbott reagents and the dif-

Table 4.—Percent Agreement With Expected Result Reported by Participants Using WBs to Detect Antibody to HIV-1*

		Reported Result		
Year	Expected Result	Nonreactive	Indeterminate	Reactive
7-85	Nonreactive (18)†	94.9	. . .	5.1
9-87	Reactive (27)	2.5	. . .	97.5
10-87	Nonreactive (15)	92.4	7.2	0.4
4-89	Reactive (20)	0.6	17.0	82.4

*WB indicates Western blot; HIV-1, human immunodeficiency virus type 1.
†Number of samples is given in parentheses throughout.

Table 5.—WB Results Obtained With Reagents From Various Manufacturers (WA-1988 to WA-1989)*

	%†					
	Nonreactive (10)‡			Reactive (15)‡		
Manufacturer	Nonreactive	Indeterminate	Reactive	Nonreactive	Indeterminate	Reactive
Bio-Rad	85.3	12.5	2.2	0.7	26.1	73.2
DuPont	88.4	10.8	0.8	0.9	29.3	69.8
Organon Teknika	95.9	4.1	0.0	0.3	9.5	90.2
In house	98.4	1.6	0.0	1.4	9.9	88.7

*WA-1988 to WA-1989 represents a time period that spans five panels.
†Data are given as percentages of results reported by participants.
‡Number of samples on the five panels.

Table 6.—Results of Donor Testing for Antibody to HIV-1*

	Reactivity, %†		
Year	**Initial**	**Repeatable**	**Confirmed**
1985	0.85	0.26	...
	0.57	0.17	...
	1.00	0.33	0.04
1986	0.50	0.10	...
1987	0.20	<0.10	...
1988	<0.10	<0.10	<0.01

*HIV-1 indicates human immunodeficiency virus type 1.
†Percentage of donors tested by responding participants.

ference in reactivity seen when samples are replicated on different panels (Table 3) suggest some of the problem is attributable to the reagent system. Although some of the lack of specificity might be due to the methods used to select and prepare the AABB/CAP survey samples, similar specificity problems have been observed with nonreactive samples included on laboratory evaluation panels sent out by the Centers for Disease Control[14] and by the American Association of Bioanalysts. On an American Association of Bioanalysts panel mailed in early 1989, four nonreactive samples were not evaluated because the majority of participants who used Abbott reagents reported them as reactive.

Panel samples that contain antibody to HIV-1 are selected to represent levels of reactivity that would be found in donor samples. Thus, testing of survey samples is not usually a good measure of the sensitivity of the reagent systems. Two recent samples, W-26 and W-41 (1988), were inadvertently diluted several fold greater than called for in the sample specifications. The EIA test results on these highly diluted samples and all other reactive samples indicate that these tests have good sensitivity. From the results on these samples, there is evidence that in some laboratories borderline reactivity may not be detected with some manufacturers' reagent systems. This could be a reagent problem; however, more than 60% of laboratories that used the same reagent system reported the samples as reactive. This suggests that performance and technical factors, as well as reagent sensitivity, are critical in the detection of low levels of antibody to HIV-1.

Evaluation of participant results with Western blots indicates that this method shows high levels of specificity and sensitivity. Overall, less than 1% of nonreactive samples were classified as reactive, and less than 2% of reactive samples were reported as nonreactive. On samples W-25 and W-44 (1988), only 4 (1.4%) of 280 reports failed to identify one or more viral bands. Recent surveys (Table 5) indicate that one problem with Western blots is the various interpretive criteria applied.[15-17]

Up to 30% of participants who used the licensed Western blot system made by Du Pont Pharmaceuticals, Wilmington, Del, interpreted reactive panel samples as indeterminate. This failure to report as reactive samples containing antibody to HIV-1 may relate to the manufacturer's rigid criteria for interpretation that were established by the Food and Drug Administration for donor reentry. Also, the difference in the staining intensity of the panel samples when compared with the controls may account for some of the discrepancy. Results that have been reported by users of other reagents have suggested that similar problems are occurring.

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Human Immunodeficiency Virus Type 1 Reference Materials

Thomas A. O'Brien, PhD

• Since licensure of the first human immunodeficiency virus type 1 screening assay in March 1985, there has been a need for well-characterized human immunodeficiency virus type 1 reference sera. These sera would be used in an effort to ensure uniformity of human immunodeficiency virus type 1 test results worldwide. Three major organizations—the World Health Organization, the Pan American Health Organization, and the National Committee for Clinical Laboratory Standards working with the College of American Pathologists—have ongoing programs aimed at the preparation of these reference sera. All three organizations are preparing both negative and human immunodeficiency virus type 1 seropositive sera. In addition, one organization is preparing early seroconverter and late acquired immunodeficiency syndrome samples. The status of these efforts and the serologic characteristics of the sera were reviewed.

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We present an update on the current efforts toward the preparation of human immunodeficiency virus type 1 (HIV-1) reference materials. The goal of these efforts is to ensure the uniformity of HIV-1 testing nationwide and hopefully worldwide. There are three major organizations—the World Health Organization (WHO), followed by the Pan American Health Organization (PAHO), and then finally, in more detail, the efforts of the National Committee for Clinical Laboratory Standards (NCCLS)—working toward standardization of HIV testing. This article will review first the efforts of these organizations. The NCCLS efforts are most relevant to the current efforts at standardization of HIV-1 testing in the United States.

WHO

In 1988, the WHO prepared and made available two HIV reference sera.[1] The first is a reactive serum identified as 86/6302. This serum is strongly reactive in nine different HIV-1 enzyme-linked immunosorbent assays (ELISAs). It is a typical reactive serum that shows core protein (*gag*), polymerase (*pol*), and envelope (*env*) polypeptides on Western blot (Fig 1).[2-4]

In addition, the WHO has a nonreactive sample, 86/6238. This sample is nonreactive by all ELISAs and by Western blot. These sera are available as freeze-dried preparations from the National Institute for Biological Standards and Control, in London, England. These materials have been available for approximately 1 year, but for unknown reasons, they have not been widely used in the United States.

The primary effort of WHO toward HIV-1 standardization is called the AIDS (acquired immunodeficiency syndrome) Reagent Project (J. Esparza, oral communication, July 1989). The goal of the AIDS Reagent Project is standardization of reagents, as well as standardization of the procedures that are used with those reagents. The AIDS Reagent Project is made up of three WHO collaborating centers on AIDS. These are the National Institute of Biological Standards and Control in London, the Institute Pasteur in Paris, France, and the National Institute of Allergy and Infectious Diseases at the National Institutes of Health in Bethesda, Md. All three WHO collaborating centers serve as repositories for research reagents. Among these research reagents are virus strains and molecular clones, polyclonal immune animal sera, monoclonal antibodies and cell lines, as well as the HIV-1 reference antibody-positive and -negative human sera discussed above. Requests for reagents from the three repositories are

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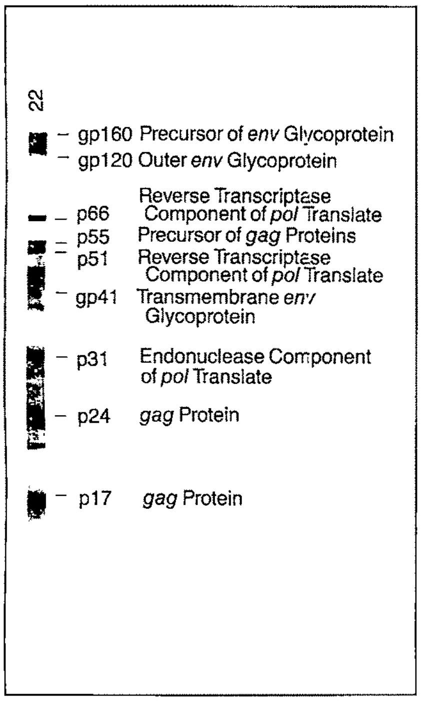
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gp160 Precursor of *env* Glycoprotein
gp120 Outer *env* Glycoprotein
p66 Reverse Transcriptase Component of *pol* Translate
p55 Precursor of *gag* Proteins
p51 Reverse Transcriptase Component of *pol* Translate
gp41 Transmembrane *env* Glycoprotein
p31 Endonuclease Component of *pol* Translate
p24 *gag* Protein
p17 *gag* Protein

Fig 1.—Western blot pattern for established seropositive sample. Major human immunodeficiency virus type 1 viral antigens are identified by their molecular weight on the Western blot strip. *env* indicates envelope polypeptides; *pol*, polymerase polypeptides; and *gag*, core protein polypeptides.

evaluated and coordinated by the WHO Global Program on AIDS in Geneva, Switzerland.

The AIDS Reagent Project is also working actively toward standardization of the laboratory techniques that are used with the research reagents in its repositories. To help new laboratories carry out these techniques, the WHO has planned and carried out a number of workshops. Workshops in 1989, for example, covered HIV isolation and characterization, *pol* chain reaction technology, and HIV monoclonal antibodies. Assays for measuring viral infectivity and neutralizing antibody will be covered in workshops held in 1990. To summarize the efforts of the WHO, the organization has taken the approach of making reference sera available quickly, and these sera have been available for about a year. The WHO are now focusing its efforts on the WHO AIDS Reagent Project that is aimed at characterizing reagents for research purposes and carrying out technical training for laboratories around the world.

PAHO

The PAHO is working on written guidelines for the evaluation and standardization of HIV-1 antibody test kits (C. Bianco, MD, oral communication, July 1989). This is a broad effort, with the preparation of HIV-1 reference materials being only one of the organization's goals. The PAHO document will contain guidelines pertaining to both HIV-1 screening and confirmatory assays. Among the topics that will be included in the sections that cover screening assays are test selection criteria, monitoring of lot-to-lot variations, and monitoring of test proficiency in the local laboratories. The ability to test laboratory proficiency is especially important. The PAHO guidelines will also discuss standardization of the Western blot confirmatory assay.

Specifically with regard to HIV-1 reference materials, the intention of the PAHO is that each country prepare its own seropositive reference standard and make it available to laboratories in that country. Ideally, each country will use the WHO reference positive serum to characterize its own country's reference standard. The PAHO specifies that the reference standard should show moderate ELISA reactivity and should contain antibodies to all the *gag*, *pol*, and *env* polypeptides on Western blot. The PAHO suggests that the country establish an expected mean optical density for the sample as a target value that would give people a measure of their proficiency in running the HIV-1 screening assays. Finally, the PAHO suggests that each local laboratory use the reference standard on every ELISA plate run, as a means of testing a known sample along with the other samples that they are testing. The reactivity of the known positive sample would be used as an additional measure of the validity of the results obtained from the ELISA plate.

NCCLS

This section of the article will review the efforts of the NCCLS in the preparation of HIV-1 reference materials. It will first discuss the composition and mission of the NCCLS and then describe, in some detail, the efforts of the HIV-1 Subcommittee. The NCCLS is a consensus system of government, professional organizations, and industry. The purpose of the NCCLS is to bring together people from a variety of backgrounds to work under the umbrella of the NCCLS for a common goal. The NCCLS has been in existence for more than 20 years. The easiest way to describe the NCCLS is to present its mission as follows:

> The National Committee for Clinical Laboratory Standards promotes medically useful testing in a clinical laboratory and alternate sites by maintaining a forum for communication and education with professionals in health care practice, government and industry and the consumers of testing products and services; operating a consensus mechanism for development, evaluation, and approval of national and international voluntary standards and guidelines; cooperating with other national and international organizations in developing, approving and implementing voluntary consensus standards and guidelines.[5]

The key word here is "voluntary." Through the NCCLS, people come together in a voluntary effort to develop standards that are then used voluntarily by NCCLS member organizations worldwide. The NCCLS carries out its mission through the use of nine area committees that cover most areas of interest in laboratory testing. The current committees are Clinical Chemistry, Evaluation Protocols, General Laboratory Practices, Hematology, Immunology and Ligand Assay, which the HIV-1 Subcommittee is part of, Instrument Systems, Microbiology, Toxicology and Drug Monitoring, and finally the National Reference System for the Clinical Laboratory. The NCCLS committees cover all major laboratory disciplines. Within each one of these area committees, there are groups of subcommittees that cover specific topics in much more detail.

NCCLS Documents

The products of the NCCLS are written documents. These documents cover a variety of laboratory-related topics, such as "Selecting and Evaluating a Referral Laboratory," "Assessment of Clinical Sensitivity and Specificity of

Laboratory Tests," and "Protection of Laboratory Workers From Infectious Disease Transmitted by Blood and Tissue." The NCCLS publishes these documents and guidelines; laboratories then use them in their own setting to run their organization. The NCCLS develops its documents through the consensus process. There are four levels in this process.[6] The documents mentioned above started out as proposed standards. These proposed standards are written by a subcommittee of experts who work on a specific topic. After the subcommittee approves a document, it is then voted on by the area committee and subsequently by the Board of Directors of the NCCLS. On approval by the Board, the document is published as a proposed standard and sent to all member organizations for a minimum 60-day review. At the end of the review, comments from the member organizations are addressed by the appropriate subcommittee, and the standard or guideline is modified to reflect those comments. Once the comments are addressed, a tentative standard is written. The tentative standard is then published and made available for review for 1 year. As with the proposed document, comments from the member organizations are reviewed, answered, and made part of the document. The document is modified as necessary, and a candidate standard for approval is prepared. After a 60-day review and voting by all active NCCLS members, a final approved standard is published. Finally, the approved standard is reviewed every 3 years to see if it is still relevant and to see if it needs to be changed.

NCCLS HIV-1 Subcommittee

The objective of the NCCLS HIV-1 Subcommittee was to write guidelines for the preparation of HIV-1 reference materials and to collaborate in their preparation and distribution. The goal of this subcommittee was not just to write a document. The subcommittee wanted to go a step further and make sure these reference materials were prepared and made available to the community as soon as possible. By working with the College of American Pathologists (CAP, Northfield, Ill), the subcommittee has made much progress in this effort, and the NCCLS and CAP are moving rapidly toward making these materials available.

The secondary objective of the subcommittee was to keep aware of other efforts on HIV-1 standardization by having a broad representation of membership. The goal was to be aware of other efforts, to collaborate in those efforts, and to try to make the HIV-1 materials available as quickly as possible. To meet these goals, it was important to have a broad representation of membership. Indeed, it has been this broad membership that has allowed the development of a truly consensus document.

NCCLS HIV-1 Subcommittee Membership*

Member	Organization
Thomas A. O'Brien, PhD (chairholder)	Ortho Diagnostic Systems Inc, Raritan, NJ
Celso Bianco, MD	New York (NY) Blood Center
Judith Britz, PhD	Becton-Dickinson & Company, Baltimore, Md
Roger Y. Dodd, PhD	American Red Cross, Rockville, Md
Jay Epstein, MD	FDA Office of Biologics Research and Review,
Alfred J. Saah, MD, MPH	The Johns Hopkins University, Baltimore, Md
Charles A. Schable, MS	Centers for Disease Control, Atlanta, Ga
W. Michael Tregellas, MS, MT, SBB	The Paragon Group, Mesa, Ariz
Judith Wilber, PhD	California Department of Health, Berkeley,Calif

*NCCLS indicates National Committee for Clinical Laboratory Standards; HIV-1, human immunodeficiency virus type 1; and FDA, Food and Drug Administration.

The Table contains the membership of the HIV-1 Subcommittee. Individuals were chosen for membership in the subcommittee, based on their own knowledge of HIV-1 testing along with the potential that their organization could help in the preparation of the materials. It is clear that the subcommittee does have the desired broad membership with representatives from government who regulate the assays, from industry who make the assays, as well as from the blood-banking, public health, and AIDS research communities.

HIV-1 Guideline

The proposed guideline was published in June 1988 and was under membership review through January 1989. The subcommittee met in March 1989 to review the comments and to draft the tentative guideline. The tentative guideline has been voted on and approved by both the HIV-1 Subcommittee and the Area Committee on Immunology and Ligand Assay. It should obtain final Board approval and be published early in 1990.

The title of the proposed guideline is "Human Immunodeficiency Virus Reference Material Specifications: Procedures for Preparing Four Human Immunodeficiency Virus Antibody Reference Materials From Human Serum."[7] The document begins with an introduction that presents the goal of this guideline and why it was put together, followed by the scope of what is covered in the guideline. Next, the sources and characteristics of the sera and plasma used for the reference materials are discussed in detail. The document then covers the collection and storage of the sera, the procedures for preparing the HIV-1 reference materials, and finally the characterization of the reference materials.

The NCCLS HIV-1 guideline covers four reference materials: (1) a negative sample, (2) an established seropositive sample, (3) an early seroconverter sample, and (4) a late AIDS sample. The subcommittee believed early on that the negative and the established seropositive samples would be the easiest to find and would be used most frequently. The early seroconverter and late AIDS samples would be more of a challenge to prepare and would be used primarily for research purposes.

Negative

Sample.—The guideline specifies that the negative serum should be collected from a low-risk donor. It should be negative by using all licensed screening tests and by Western blot, by immunofluorescence, and by radioimmunoprecipitation. Both the initial sample and a subsequent follow-up sample must be negative by all the assays noted

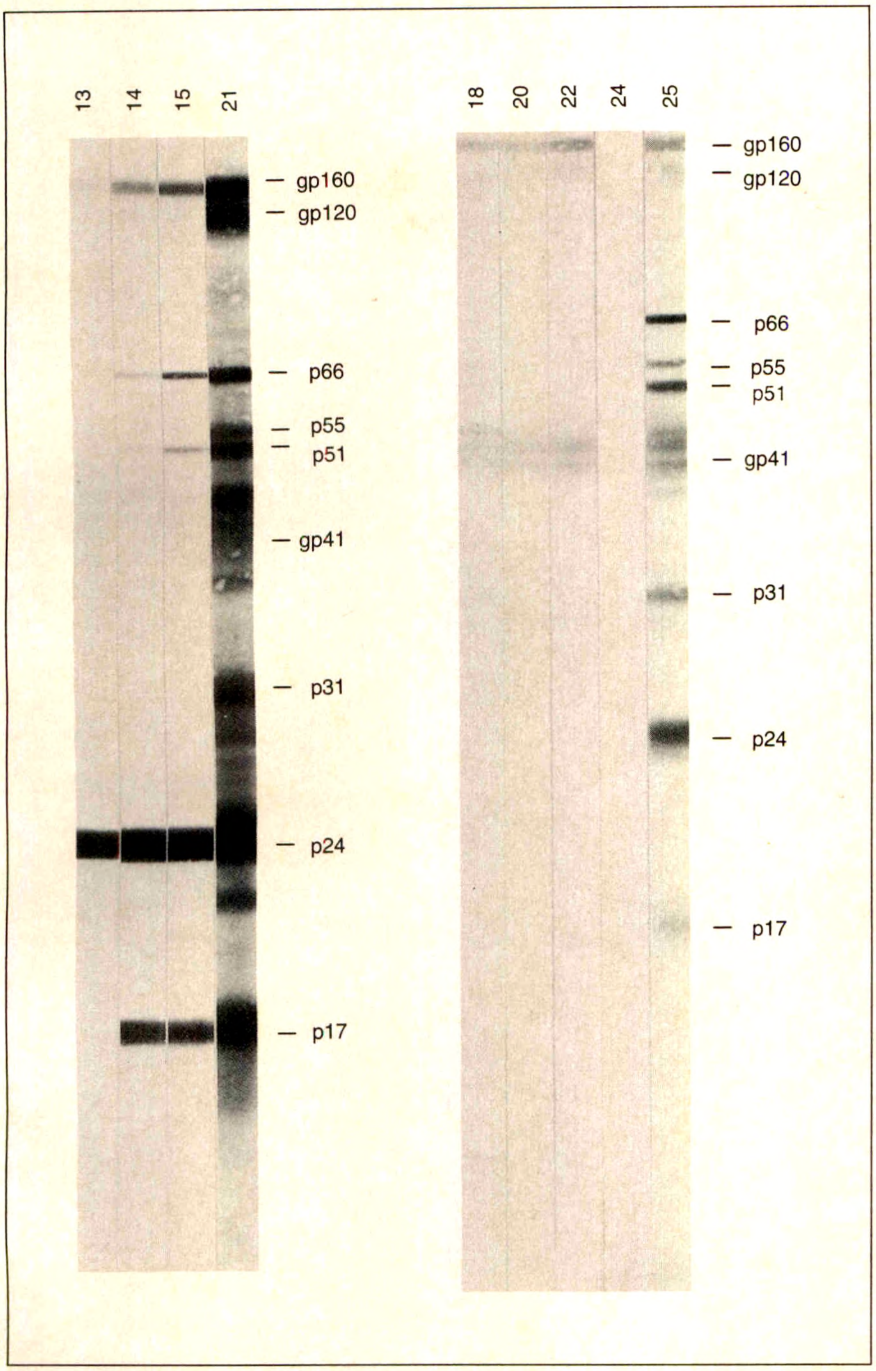

Fig 2.—Western blot patterns for early seroconverter and late acquired immunodeficiency syndrome samples. Left, Early seroconverter: strip 13, day 1 (first sample with human immunodeficiency virus type 1 antibodies); strip 14, day 14; strip 15, day 35; and strip 21, human immunodeficiency virus type 1 seropositive control. Right, Late acquired immunodeficiency syndrome: strips 18, 20, and 22 contain various late acquired immunodeficiency syndrome samples; strip 24, negative control; and strip 25, human immunodeficiency virus type 1 seropositive control.

above. If the subsequent sample is negative, then the first sample donation is acceptable for use in preparing the reference material.

Established Seropositive Sample.—The established seropositive sample is similar to the WHO and PAHO materials. The established seropositive sample should be positive by using all licensed screening assays and by Western blot, immunofluorescence, and radioimmunoprecipitation. On Western blot all *gag*, *pol*, and *env* polypeptides should be present. Figure 1 shows the Western blot pattern of the proposed seropositive sample.[4] All the major HIV bands are present: the *gag* bands at p17, p24, and p55, the *pol* bands at p31, p51, and p66, and the *env* bands at gp41 and gp120/160. This is typical of what the seropositive material should look like.[2,3]

Early Seroconverter

Sample.—The early seroconverter should be a sample early after infection but positive by all licensed screening assays. In specifying this material, the subcommittee did not want to specify a material that would be used to characterize the relative sensitivities of assays. The purpose of the material will be to give a measure of laboratory proficiency, not for comparing assays, thus the requirement that it be positive by all licensed screening assays. This may make it more of a challenge to find the sample but that is the requirement that is in the document.

By using a licensed Western blot, the sample should show reactivity with p24 and gp120/160, with no more than a trace reactivity of gp41.[2,3] A follow-up sample should be obtained within 2 months of the initial sample, and this sample should show progressive seroconversion on the licensed Western blot, immunofluorescence, and radioimmunoprecipitation. If the follow-up sample does show a pattern typical of seroprogression, the first sample is then acceptable for use in preparing the reference material.

Figure 2, left, shows a typical seroconverter sample that would meet the specifications in the guidelines.[4] Lane 13 is an early seroconverter sample that shows p24 and gp120/160 bands on day 1. The next lane 14 (day 14) shows bands at p17 and p24, as well as a more intense gp120/160 band. Finally, the last sample (day 35) shows a further increase in intensity of the p17, p24, and gp120/160 bands. Based on this seroprogression, sample 13 would be considered adequate for the early seroconverter sample.

Late AIDS.—This sample is collected from a patient with AIDS as defined by the Centers for Disease Control, Atlanta, Ga. As with the seroconverter, it should be positive by all licensed screening assays. On the licensed Western blot, it should show primarily envelope reactivity with no more than a trace of p24 reactivity. Figure 2, right, shows the Western blot pattern of acceptable late AIDS samples that show gp41 and gp120/160 reactivity in lanes 18 through 22.[4] Typical of late AIDS sera, there is only a trace, if any, of p24 reactivity.

CAP HIV-1 Reference Materials

As was mentioned in the beginning of this article, the NCCLS has been working closely with the CAP in an effort to make the four reference sera available to the public as soon as possible. The CAP is putting together two sets of reference materials: (1) a screening set which contains the negative and the established seropositive samples and (2) a reference set that contains the early seroconverter and the late AIDS samples (D. Schapp, oral communication, July 1989). It is expected that the screening set will be used fairly widely, while the reference set will be used primarily for research purposes. The screening set will have four sample vials per set, ie, two negative vials and two seropositive vials. The reference set, because of the difficulty in finding these materials, will contain only two vials per set, ie, one for the early seroconverter and one for the late AIDS sample.

CONCLUSION

We discussed efforts by the WHO, PAHO, and NCCLS to prepare HIV-1 reference materials. WHO has had positive and negative reference materials available for more than a year. Although these WHO materials have been available, they have not been widely used in the United States. To complement the use of these sera, WHO is preparing standardized research reagents and training materials for use in laboratories worldwide. The PAHO is early in its efforts. The PAHO has written preliminary guidelines that recommend the use of WHO positive standard in preparing each country's own reference standard. Finally, the NCCLS HIV-1 Subcommittee has written guidelines for the preparation of four HIV-1 reference materials. These include negative, seropositive, early seroconverter, and late AIDS sera. This effort is novel in that for the first time representatives from many of the organizations involved in HIV-1 testing in the United States came together and developed a guideline with wide consensus agreement. The CAP is working rapidly to make these materials available.

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Role of the Microbiology Laboratory in the Diagnosis of Opportunistic Infections in Persons Infected With Human Immunodeficiency Virus

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• Patients with defects in the immune system caused by infection with human immunodeficiency virus are predisposed to a variety of opportunistic infections, many of which can be diagnosed by conventional or special microbiological procedures. This article reviews the laboratory diagnosis of bloodstream, lower respiratory tract, gastrointestional tract, and central nervous system infections in persons infected with human immunodeficiency virus. Laboratories serving health care facilities in which a large number of patients with human immunodeficiency virus infection are cared for should consider enhancing the detection of the specific pathogens affecting these patients by the use of special procedures, including blood culture methods for mycobacteria and fungi, examination of induced sputum for *Pneumocystis carinii*, and modified acid-fast stains on feces to identify *Cryptosporidium* and *Isospora belli*.

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The multiple defects in the normal host immune system caused by infection with human immunodeficiency virus (HIV) predispose to an increased frequency of a variety of opportunistic infections caused by bacteria, mycobacteria, fungi, protozoa, and viruses. The infecting organisms are generally obligate or facultatively intracellular parasites capable of causing latent infections. Many of the infectious complications of the acquired immunodeficiency syndrome (AIDS) are reactivations of latent infections acquired in the distant past. Frequently, treatment of these organisms must be prolonged, often for months or even for life.

The primary immunologic defect in these patients is depletion of the T-helper lymphocytes. The occurrence of infections with certain microorganisms in HIV-infected persons signals the development of clinically significant immunologic dysfunction; the definitive laboratory diagnosis of the opportunistic infections discussed herein in patients without a known cause of immunodeficiency (with or without laboratory evidence of HIV infection) usually suggests a diagnosis of AIDS.

B-cell dysfunction is a secondary consequence of T-helper lymphocyte depletion; patients with HIV infection frequently show an impaired ability to develop a primary or an anamnestic antibody response to challenge with new or recall antigens. This defect limits the usefulness of serologic diagnosis in patients with AIDS and also limits the efficacy of polysaccharide vaccines, such as the pneumococcal vaccine.[1] Table 1 lists the predominant infectious disease complications of HIV infection and their relation to T- and B-cell defects.

In most cases, the laboratory processing of specimens collected from HIV-infected individuals is the same as for specimens from other patients. However, special procedures can enhance detection of certain microorganisms in the bloodstream, lower respiratory tract, gastrointestinal tract, or central nervous system when the laboratory examines specimens submitted from HIV-infected patients, taking into consideration the pathogens found with increased frequency in this group of patients.

BLOODSTREAM INFECTIONS

The general principles of optimal blood culture practice—collecting high-volume blood cultures in adults and collecting two or three blood cultures per septic episode—are just as important in HIV-infected patients as in non–HIV-infected patients.[2] Several studies have documented that recovery of bacteria from blood is related directly to the volume of blood cultured; thus, it is recommended strongly that 20 to 30 mL of blood should be collected per culture in adults. Even when high-volume blood cultures are collected, the number of cultures cannot be reduced to fewer than two per 24 hours, because some bacteremias are intermittent. It is also helpful to demonstrate bacteremia in more than one culture to confirm the

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Table 1.—Frequent Infectious Complications

- Infections reflecting T-cell defects
  - Parasites
    - *Pneumocystis carinii*
    - *Toxoplasma gondii*
    - *Crytosporidium*
    - *Isospora belli*
  - Bacteria
    - *Mycobacterium avium-intracellulare*
    - *Mycobacterium tuberculosis*
    - *Salmonella* species
    - *Legionella* species
    - *Nocardia asteroides*
    - *Listeria monocytogenes*
  - Fungi
    - *Candida albicans*
    - *Cryptococcus neoformans*
    - *Histoplasma capsulatum*
    - *Coccidioides immitis*
  - Viruses
    - Cytomegalovirus
    - Herpes simplex
    - Varicella zoster
    - Papovavirus (JC virus)
- Infections reflecting B-cell defects
  - *Haemophilus influenzae*
  - *Streptococcus pneumoniae*

persistence of bacteremia and to exclude contamination.

Bacteremia, mycobacteremia, and fungemia are common in patients with HIV infection, especially in those with AIDS and AIDS-related complex (ARC). The spectrum of organisms recovered from HIV-infected patients is significantly different from that seen in other types of patients. Whimbey et al[3] at Memorial Hospital in New York, NY, reported that 49 (14.6%) of 336 patients with AIDS seen from January 1980 to March 1985 had bloodstream infection with *Mycobacterium avium–intracellulare*, an organism that is rarely recovered from other patient groups, and that 38 (11.3%) had nonmycobacterial bacteremia or fungemia. The most common organisms recovered in the latter group were *Cryptococcus neoformans* (in 6 patients), *Candida albicans* (in 4), *Staphylococcus aureus* (in 9), and *Salmonella* species (in 7). A study from San Francisco (Calif) General Hospital[4] also reported a high incidence of *S aureus* bacteremia in patients with AIDS and ARC. None of these patients had neutropenia, a recent history of intravenous drug abuse, or lymphedema secondary to Kaposi's sarcoma, suggesting the importance of *S aureus* as a pathogen in this patient population, even without those factors that predispose to staphylococcal infection. *Salmonella*-infected patients with early AIDS may present with bacteremia that relapses despite appropriate antibiotic therapy. In the general population, approximately 5% of persons with nontyphoidal salmonellosis become bacteremic. In contrast, a review of *Salmonella* infection associated with AIDS showed that 52 (78%) of 67 patients with AIDS and salmonellosis had bacteremia—15 times the frequency in the general population.[5]

MYCOBACTERIAL METHODS

Although there has been evidence of mycobacteremia occurring in many forms of mycobacterial disease since the late 19th century,[6] laboratory techniques for the recovery of mycobacteria from blood have been little used in recent years. The dramatic appearance of AIDS and the associated bacteremia due to *M avium–intracellulare* have renewed interest in mycobacterial blood cultures; there has also been some evidence of an association of AIDS and bacteremia due to *Mycobacterium fortuitum*, *Mycobacterium chelonei*, and *Mycobacterium tuberculosis*, although *M tuberculosis* is uncommonly recovered from blood and is usually identified in respiratory secretions or tissue.

Infection with *M avium–intracellulare* is a late complication in many patients with AIDS, with a median survival from the time of diagnosis of 3 months.[1] Symptoms are often surprisingly mild, despite bacteremia, and may include fever, cramping abdominal pain, and diarrhea. Massively enlarged mesenteric lymph nodes are often present and may be palpable. Blood cultures are the most sensitive diagnostic procedure and are positive in nearly all cases.

Procedures designed specifically for mycobacteria are necessary to maximize recovery of these organisms from the blood. Two methods have proved particularly effective: the Isolator lysis-centrifugation system and the Bactec radiometric method. Macher et al[7] used the Isolator system to prepare a concentrate from the blood specimen, which was inoculated into Lowenstein-Jensen, Middlebrook 7H10, and Sabouraud's agar media. Positive cultures were detected after 14 to 51 days of incubation. Direct inoculation of a radiolabeled Middlebrook medium (13A) that is tested on the Bactec 460 has recently been introduced. Strand et al[8] compared the overall sensitivity and time to detection of the Bactec 13A medium and water lysis-centrifugation. Recovery rates were 96.9% and 98.5%, and the mean times to detection were essentially the same (23.6 and 23.8 days), but the direct inoculation method avoids the time-consuming manual manipulations required by lysis-centrifugation.

FUNGAL METHODS

Although fungemia is quite common in HIV-infected patients, many blood culture procedures lack sensitivity for recovering these organisms; thus, negative blood cultures may not rule out disseminated candidiasis, cryptococcosis, or other mycotic diseases. *Cumitech 1A: Blood Cultures II*[9] recommends the following three procedures for fungal blood cultures: (1) brain-heart infusion broth or a biphasic brain-heart infusion medium system; (2) the Bactec radiometric system (however, routine blind subcultures are necessary); and (3) the Isolator lysis-centrifugation method. The latter is generally considered optimal. Between 1983 and 1985, 10 studies[10] compared the Isolator method with different broth and biphasic media blood culture systems. In 5 of these studies, the Isolator method showed significantly increased recovery of fungi; no difference was found in the remainder. One of the latter reports[11] compared Isolator and Bactec, the most widely used blood-culture system, and found Isolator to be superior in the recovery of the yeasts *Cryptococcus*, *Rhodotorula*, and *Torulopsis*. Ten of 14 isolates of *Cryptococcus* species were recovered only by the Isolator method, compared with the Bactec aerobic and anaerobic radiometric media ($P<.01$). Since *Cryptococcus* is a relatively common pathogen in HIV-infected patients, the Isolator system is recommended for fungal blood cultures in this patient population.

Since certain microorganisms cause frequently bloodstream infections in HIV-infected patients than in other types of patients, laboratories that serve large numbers of HIV-infected patients should employ blood culture procedures that are effective in recovering these organisms. These laboratories might offer a general blood culture procedure designed to recover the majority of bacteria and yeasts to meet most blood culturing needs, as well as special blood culture methods for patients suspected of having bloodstream infections with mycobacteria or fungi (Table 2). The Isolator system can efficiently recover most ordinary bacteria, as well as mycobacteria and fungi, and is an excellent system to employ in HIV-infected patients. However, because the Isolator method shows poor recovery of anaerobes and streptococci,[10] it should not be used alone but should be combined with a nonvented broth bottle, which will effectively recover the latter two organism groups.

Table 2.—Blood Culture Techniques

- General (routine) blood culture
  - Broth culture with visual inspection and early blind subculture
  - Broth culture with visual inspection and carbon dioxide detection
  - Lysis-centrifugation (Isolator*) and nonvented broth
  - Other systems
- Fungal blood culture
  - Lysis-centrifugation (Isolator) (this is optimal method)
  - Biphasic brain-heart infusion medium
  - Carbon dioxide detection method (blind subculture is necessary)
- Mycobacterial blood culture
  - Lysis-centrifugation (Isolator)
  - Carbon dioxide detection using Bactec 13A broth

*If Isolator is used as general blood culture method, a nonvented broth medium must be included for each blood culture, because Isolator alone is a suboptimal method for recovery of anaerobes and streptococci.

LOWER RESPIRATORY TRACT INFECTIONS

Table 3 shows the pulmonary infections that may occur in HIV infection. There is an increased incidence of bacterial pneumonia in HIV-seropositive individuals; in patients with AIDS these pneumonias are commonly caused by *Streptococcus pneumoniae, Haemophilus influenzae*, and *S aureus*.[12] Pyogenic bacterial pneumonias caused by *S pneumoniae* or *H influenzae* may be patchy or diffuse on chest roentgenograms and indistinguishable from *Pneumocystis carinii* or cytomegalovirus pneumonitis. Simultaneous infections with *P carinii* and bacteria or other organisms are not uncommon. If patients with HIV infection or members of high-risk groups present with clinical findings suggestive of a pyogenic lower respiratory tract infection (respiratory symptoms and/or an abnormal chest roentgenogram showing lobar consolidation), a sputum specimen should be examined by Gram's stain and culture, and two or three blood cultures should be collected. The latter procedure is particularly important because the frequency of bacteremia in HIV-infected patients with bacterial pneumonia is high. If these specimens are negative for pyogenic bacteria, then diagnostic strategy should be targeted to *P carinii*, which is by far the most common serious infectious disease in HIV-infected individuals.

Table 3.—Pulmonary Infections

- *Pneumocystis carinii* pneumonia
- Bacterial pneumonia
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Staphylococcus aureus*
- Cryptococcosis
- Disseminated *Mycobacterium avium* complex or *Mycobacterium kansasii**
- Tuberculosis
- Cytomegalovirus pneumonia
- Toxoplasmosis
- Strongyloidiasis
- Bronchopulmonary candidiasis
- Disseminated histoplasmosis*
- Disseminated coccidioidomycosis*
- Herpes simplex pneumonia
- Cryptosporidiosis

*Disseminated at a site other than, or in addition to, lungs and cervical or hilar lymph nodes.

The laboratory diagnosis of *P carinii* pneumonia is extremely important in HIV infection, since this treatable disease is the presenting manifestation in 60% of patients with AIDS, and more than 80% of them will develop it at some time during their clinical course.[13] There is a high frequency of relapse; 20% to 30% of patients will have a second episode, and three or even more episodes may occur. Characteristically, *P carinii* pneumonia presents with progressive shortness of breath, cough (often not prominent), and fever, and the chest roentgenogram shows bilateral interstitial-alveolar infiltrates. Presentation is often atypical in HIV-infected patients, and a very high index of suspicion is required. For example, chest roentgenograms are often normal or nearly normal, and atypical presentations include nodular disease, cavitary disease, and pneumothorax. Diagnosis may be difficult in patients receiving aerosol pentamidine prophylaxis, who may have a modified illness with only upper lobe involvement.

Laboratory diagnosis depends on the demonstration of the characteristic cysts and/or trophozoites in respiratory secretions or in pulmonary tissue obtained by biopsy. Although bronchoscopy with bronchoalveolar lavage and biopsy—the method most frequently employed to establish the diagnosis—has been found to be a safe and sensitive procedure, there is the potential for complications and the procedure is relatively costly.

Examination of ordinary expectorated sputum is not useful for diagnosis of pneumocystosis, and before the advent of AIDS, *P carinii* pneumonia was rarely diagnosed by sputum examination. In a retrospective review of 194 cases of *P carinii* pneumonia in patients without AIDS diagnosed between 1969 and 1972, it was estimated that the diagnostic yield for sputum was 6%.[14] However, in 1986 two important studies[15,16] were published showing that *P carinii* can be detected in 50% to 60% of infected patients with AIDS by methenamine-silver or Giemsa-type staining of sputum induced by an aerosol of hypertonic saline.

The induced sputum is mucolysed, concentrated by centrifugation, and stained by a rapid modified Giemsa stain. When induced sputum was the first diagnostic specimen examined in 404 episodes of suspected HIV-associated pulmonary disease in 358 patients, *P carinii* was found by this method in 222 (55%) of specimens. The range of sensitivity for detection of *P carinii* in induced sputum was 74% to 77%, with a negative predictive value of 58% to 64%.[17] To achieve this relatively high sensitivity, the procedure for sputum induction and processing must be carefully followed.[13] This induced sputum examination approach minimizes patient discomfort and reduces cost by limiting the number of bronchoscopies performed to those patients whose induced sputum examinations do not show *P carinii*. An algorithm for the diagnosis of pulmonary infections in HIV-infected patients has been developed and is now in use at San Francisco General Hospital.[17] However, the yield of mycobacteria and fungi from culturing induced sputum specimens was low compared with that from bronchoalveolar lavage specimens. It is not known whether these differences will be seen in other patient groups, particularly in populations with a high incidence of pulmonary tuberculosis.

The ability to detect *P carinii* is even better when indirect immunofluorescence with monoclonal antibodies is used. In one institution,[18] immunofluorescence detected 45 (92%) of 49 cases of *P carinii* pneumonia, whereas Diff-Quik and toluidine blue stains detected 37 (76%) and 39 (80%) cases, respectively. The increased sensitivity of immunofluorescence with monoclonal antibodies is probably related to both the improved screening efficiency attained with fluorescence microscopy and the high specificity of immunofluorescence staining. However, compared with toluidine blue staining, immunofluorescence does not show increased sensitivity in detecting *P carinii* in bronchoalveolar lavage specimens.

Physicians must be prudent in selecting patients truly at risk for *P carinii* pneumonia for induced sputum examination. The highest yield will be obtained for patients at risk for infection with HIV who present with a nonproductive cough and a chest roentgenogram showing diffuse interstitial markings. Induced sputum examination will have little value in patients with a purulent sputum specimen and a chest roentgenogram showing classic lobar pneumonia. In addition, other pulmonary infectious agents cannot be evaluated adequately by examination of induced sputum. If a sputum specimen is negative for *P carinii* or if a patient positive for *P carinii* detected by induced sputum examination responds poorly to therapy, bronchoscopy with biopsy and bronchoalveolar lavage should be strongly considered to assess the possible presence of other infections (or neoplasms).

Data from the National Heart, Lung and Blood Institute Workshop on the Pulmonary Complications of AIDS[19] and from San Francisco General Hospital[20] have shown that bronchoscopic procedures are very useful for detecting other opportunistic infections besides *P carinii*, especially when bronchoalveolar lavage and transbronchial biopsy are combined and when cultures for bacteria, mycobacteria, fungi, and viruses are performed. The San Francisco study showed that when the detection or recovery rates for bronchoalveolar lavage and transbronchial biopsy were considered together, the yield for all 173 pathogens in 171 patients was 98%. The organisms identified included *P carinii*, *M avium–intracellulare*, *C neoformans*, *M tuberculosis*, *Coccidioides immitis*, and *Histoplasma capsulatum*. The sensitivity of bronchoalveolar lavage alone was higher than that of transbronchial lung biopsy alone except for the detection of *C neoformans* and *P carinii*. For the diagnosis of *M avium–intracellulare*, bronchoalveolar lavage was especially valuable, although the significance of finding this organism in the lung is not fully understood. Likewise, isolation of cytomegalovirus from bronchoalveolar lavage fluid or transbronchial lung biopsy specimen may not be indicative of pneumonia caused by this organism. Many patients who have pulmonary disease and cytomegalovirus isolated from their lungs have concurrent infection with other microorganisms, especially *P carinii*. The diagnosis of cytomegalovirus pneumonia is probably best made by a positive culture from lung tissue or bronchoalveolar lavage and the presence of pathognomonic cells with intranuclear inclusion bodies in tissue or cytological preparations. In one study[21] bronchoscopy yielded the diagnosis of cytomegalovirus pulmonary infection in only 55% of autopsy-proved cases. In that series, postmortem examination showed diffuse cytomegalovirus lung infection in seven patients, but only focal infection in three others. Cytomegalovirus infection was missed by bronchoscopy in all of the cases showing only focal involvement.

From 1953 through 1984, the total number of cases of tuberculosis reported in the United States decreased by 73.6%. This decades-long pattern halted in 1985 and reversed in 1986, with the first increase (2.6%) in the number of indigenous tuberculosis since national reporting began.[22] The failure of the tuberculosis rate in the United States to continue its long pattern of decline appears at least partially attributable to HIV.

Increases in the number of cases of tuberculosis have been largely limited to the demographic groups among which the large majority of AIDS cases have also occurred. For example, in New York City, 4% of patients with AIDS had a history of tuberculosis, and in Florida, 10% of patients with AIDS had a history of tuberculosis. Tuberculosis preceding the diagnosis of AIDS in a large proportion of cases suggests that an HIV-induced immune deficiency that is not severe enough to cause the opportunistic diseases that constitute the majority of AIDS complications may allow preexisting tuberculosis infection to progress to clinical tuberculosis. Of course, factors other than HIV infection, such as poverty, homelessness, and immigration from countries with high tuberculosis prevalence, also contribute to the trend reversal of tuberculosis.

The speed of both detection and identification of *M tuberculosis* and *M avium–intracellulare* has advanced in the last decade. Using conventional isolation methods, Peterson et al[23] reported that the average detection time was 21 days for *M tuberculosis* and 28 days for *M avium–intracellulare*. The radiometric Bactec system has greatly reduced this detection time, especially for *M avium–intracellulare* species, for which positive cultures can be detected on the average in 7 to 8 days. However, once detected, these isolates can take up to 4 to 6 weeks for final identification by standard biochemical methods. Nucleic acid hybridization employing commercially available DNA probes that are complementary to ribosomal RNA (Gen-Probe, San Diego, Calif) can reduce identification of *M tuberculosis* and *M avium–intracellulare* to 2-hour liquid hybridization.

GASTROINTESTIONAL INFECTIONS

Infections of the gastrointestional tract, although usually not life-threatening, cause significant morbidity in HIV-infected patients.

Candidal oropharyngitis (thrush) occurs in 80% to 90% of patients with AIDS and ARC.[24] However, disseminated candidiasis is rare, even at autopsy. Relatively normal neutrophil function probably prevents the dissemination of *Candida*, even as the defective T-cell function predisposes to mucosal candidiasis. In some studies, oral thrush is the most frequent opportunistic infection in patients with AIDS. In one study, half of 18 individuals at risk for AIDS who developed oral candidiasis were diagnosed as having AIDS within a median time of 3 months. Patients who have oral thrush are also at particularly high risk for *P carinii* pneumonia, and patients with ARC who have oral thrush are at risk of developing bacterial pneumonias due to *S pneumoniae* or *H influenzae*.[25] The laboratory diagnosis of oral candidiasis is made by finding blastospores and pseudohyphae in smears prepared from typical plaques in the mouth and throat. Because *Candida* species can be recovered from the oral cavity of normal persons, cultures are not useful for the diagnosis.

Esophagitis in HIV-infected patients can be caused by *Candida* (14% of patients with AIDS), cytomegalovirus, and herpes simplex virus.[26] These entities are indistinguishable on clinical and radiological grounds, but a specific diagnosis can be made by endoscopy with histologic examination or viral culture of a biopsy specimen. Since specific therapy differs for each infectious agent, definitive diagnosis is important.

Enterocolitis (or enteritis or colitis) with diarrhea is a major cause of morbidity in patients with AIDS and is seen in 50% to 60% of patients with AIDS in the United States and in at least 95% of those in Africa or Haiti.[27] In many HIV-infected persons, diarrhea is associated with fever, malaise, and marked weight loss, which can be severely disabling and even fatal. Many HIV-infected homosexuals are predisposed to enteric infections as well as to traditional sexually transmitted diseases. Pathogenic gastrointestinal protozoa, bacteria, and viruses are much more common in homosexual and bisexual men than in the general population. An intensive National Institutes of Health study[28] found one or more gastrointestinal pathogens in 17 of 20 male homosexual patients with AIDS who had diarrhea; 8 of the 17 had two or more microbial agents. The most frequent identified potential pathogens were cytomegalovirus (in 9 patients), *Entamoeba histolytica* (in 5), *Cryptosporidium* (in 3), *Salmonella typhimurium* (in 3), and *Giardia lamblia* (in 3). *Campylobacter jejuni*, *Shigella flexneri*, *M avium–intracellulare*, and herpes simplex virus were each seen in 1 or 2 patients. No intestinal pathogen was identified in 3 of the 20 patients. In contrast, pathogens were identified in only 1 of 10 patients with AIDS who did not have diarrhea, suggesting that the enteric pathogens in the symptomatic patients contributed to their diarrhea.

A study of diarrhea in 179 HIV-antibody–positive patients (49 with AIDS) at St Stephen's Hospital in London, England,[29] found potential microbial pathogens in 38.5%. This result contrasts with the National Institutes of Health study[28] in which gastrointestinal pathogens (including cytomegalovirus) were identified in 17 (85%) of 20 patients with AIDS who had diarrhea. There are probably two reasons for this disparity: (1) The National Institutes of Health study used a more exhaustive diagnostic evaluation protocol (including endoscopic biopsies of duodenal and colonic mucosa and virus cell cultures) than did the London investigation, and (2) the patients in the National Institutes of Health study all had AIDS and thus were more immunologically suppressed than the patients in the St Stephen's Hospital study, only 27.4% of whom had AIDS.

Recurrent or relapsing *Salmonella* infection, often associated with bacteremia, has been described in patients with AIDS,[5,30] and chronic *Shigella* infection, persisting for almost 1 year, has been reported[31] in an HIV-infected homosexual man who subsequently developed AIDS. A few cases of *S flexneri* bacteremia have also been reported in patients with AIDS. A large number of pathogens, including classic protozoa (such as *Giardia* and *Entamoeba*), unusual protozoa (such as *Cryptosporidium* and *Isospora*), and enteric bacteria (such as *Salmonella* species, *Shigella* species, and *C jejuni*), have been detected in the intestinal tracts of these patients. Also, viruses, fungi, and helminths, as well as various noninfections diseases, can cause diarrhea in patients with AIDS. It is apparent that the number of agents capable of causing serious enteric infection in patients with AIDS has been expanding as experience with this disease increases. Table 4 lists the common infectious causes of diarrhea in patients with HIV infection.

Table 4.—Agents Causing Diarrhea in Acquired Immunodeficiency Syndrome (AIDS)

- Bacteria
    - *Salmonella* species
    - *Shigella* species
    - *Campylobacter* species
    - *Neisseria gonorrhoeae**
    - *Mycobacterium avium-intracellulare*
    - *Chlamydia trachomatis**
    - *Treponema pallidium**
- Protozoa
    - *Entamoeba histolytica*
    - *Giardia lamblia*
    - *Cryptosporidium*
    - *Isospora belli*
    - *Microsporidium* (diagnosis by electron microscopy)
- Viruses
    - Cytomegalovirus
    - Herpes simplex*
    - AIDS-associated enteropathy
- Fungi
    - *Candida albicans*

*Causes anorectal disease.

The laboratory approach to identifying most intestinal infections in HIV-infected individuals is to use standard fecal examination procedures. For example, for fecal parasitic examination,[32] collect three separate stool specimens on different days in two vials containing preservative: one containing formaldehyde solution; and one, polyvinyl alcohol fixative. A permanent stain, prepared from the polyvinyl alcohol preservative, is frequently important for the detection and correct identification of intestinal protozoa and is recommended for every stool sample submitted for examination for parasites. Standard procedures for laboratory diagnosis of bacterial diarrhea[33] should be followed with fecal specimens cultured for *Salmonella*, *Shigella*, and *C jejuni*.

Of course, there are special procedures that are appropriate in examining fecal specimens for specific parasites that may be present in HIV infection, eg, the coccidian organisms *Cryptosporidium* and *Isospora belli*. These organisms are detected in the microbiology laboratory by identification of the oocyst stage of the parasite in feces. Oocysts are usually identified by acid-fast staining of fecal smears or concentrates with carbolfuchsin (cold Kinyoun or Ziehl-Neelsen stain), by fluorescence staining with auramine-rhodamine, by oocyst flotation on Sheather's sucrose solution, or by immunofluorescence detection of the oocysts. It is important to recognize that during the routine fecal parasite microscopic examination of the iodine stain of material preserved in formaldehyde solution, the observation of round, colorless, refractile structures 4 to 5 μm in size is suggestive of *Cryptosporidium*. An acid-fast (or alternative *Cryptosporidium* stain) smear should be performed on these specimens and in the majority of specimens will confirm the presence of *Cryptosporidium*. Garcia et al[34] compared 15 methods (not including immunofluorescence) of oocyst detection and concluded that modified (hot) Ziehl-Neelsen staining of stool preserved in 10% formaldehyde solution was superior to the other methods. However, Garcia et al[35] later studied detection of *Cryptosporidium* oocysts in 99 positive human feces specimens by immunofluorescence using monoclonal antibodies and found the method to be both extremely sensitive and specific. Seven specimens previously negative by the hot modified acid-fast technique were positive by the monoclonal immunofluorescence procedure; these specimens had low numbers of organisms. This ability to detect low numbers of organisms may become more important when drugs that are effective in treating cryptosporidial enteritis are developed. An advantage of the immunofluorescence method, in addition to sensitivity, is that slides can be screened in considerably less time with the technique, which could significantly reduce labor costs. The immunofluorescence reagents are available commercially (Meridian Diagnostics Inc, Cincinnati, Ohio).

Table 5.—Neurologic Infectious Complications in Acquired Immunodeficiency Syndrome*

- HIV induced
  - Aseptic meningitis
  - HIV encephalopathy
  - Other
- Other viral related
  - CMV (encephalitis, retinitis, cranial neuropathy, progressive polyradiculopathy)
  - Herpes zoster virus (encephalitis, cranial neuropathy, myelitis, radiculitis)
  - Herpes simplex type I (encephalitis, cranial neuropathy)
  - Herpes simplex type II (encephalitis, cranial neuropathy, myelitis, radiculitis)
  - Papovavirus (JC virus, progressive multifocal leukoencephalopathy)
- Nonviral related
  - Protozoa
    - *Toxoplasma gondii* (cerebral abscess, encephalitis)
    - *Acanthamoeba* (very rarely)
  - Fungi
    - Crytococcus neoformans (meningitis, cryptococcoma)
    - Other (*Candida, Aspergillus, Coccioioides, Histoplasma*) (rarely)
  - Bacteria (rarely)
    - *Listeria* (meningitis, abscess)
    - *Nocardia* (abscess)
    - *Escherichia coli* (meningitis)
    - *Treponema pallidum* (neurosyphilis)
  - Mycobacteria
    - *Mycobacterium tuberculosis* (abscess, meningitis)
    - *Mycobacterium avium-intracellulare* (encephalitis, meningitis)

*HIV indicates human immunodeficiency virus; CMV, cytomegalovirus.

CENTRAL NERVOUS SYSTEM INFECTIONS

Neurologic symptoms occur in up to two thirds of all patients with AIDS during their lifetime, and most of these neurologic complications are secondary to infection of the brain or meninges.[36] Table 5 shows that the infectious causes of central nervous system disease in AIDS can be caused by viruses (including HIV), protozoa (particularly *Toxoplasma gondii*), fungi (especially *C neoformans*), bacteria, and mycobacteria.

The HIV-induced diseases and other viral diseases may show a cerebrospinal fluid (CSF) mononuclear pleocytosis and elevated protein level. Aseptic meningitis occurs in 5% to 10% of patients with AIDS, and HIV encepalopathy (or AIDS dementia) occurs in 65% or more of patients with AIDS.[36] However, the diagnosis of these viral diseases is primarily clinical, and no specific microbiological diagnosis is possible unless viral isolation, which is unusual, or molecular biology techniques, which are currently not widely available, are used.

Toxoplasma gondii is among the most prevalent causes of latent infections of the central nervous system throughout the world. *Toxoplasma* is a genus of protozoa that exist in three forms: proliferative (tachyzoite), tissue cyst, and oocyst. Only tachyzoites and cysts are found in humans, who are incidental hosts; however, each of the three forms is potentially infectious for humans. Whereas normal host immunity is sufficient to contain the latent infection (cyst form) and thereby limit disease, immunocompromised patients, such as those with AIDS, are at risk for reactivation and dissemination of a previously quiescent infection.

Cerebral toxoplasmosis is an important disease in AIDS, affecting 14% of homosexuals with the disease; it is the most common cause of focal intracerebral lesions in patients with AIDS.[36] Patients with AIDS who have previously been infected are at considerable risk for development of toxoplasmosis; at least 30% of patients with AIDS who are seropositive for *T gondii* will ultimately develop toxoplasmosis, but only 1% to 3% of patients with AIDS who are seronegative will develop this disease.[37]

Definitive diagnosis of toxoplasmic encephalitis can be made only by demonstration of the organism in brain tissue. However, because of the undesirability of brain biopsies, the vast majority of cases in the United States are presumptively diagnosed by neuroradiological studies, usually after the initial diagnosis of AIDS has been made. Wright-Giemsa stain of a cytocentrifuge preparation of a CSF specimen obtained from a patient with HIV infection may demonstrate tachyzoites.[38]

Serologic testing for *T gondii* is generally not useful in demonstrating a titer rise, because this occurs infrequently in AIDS; however, it is very valuable in predicting which patients are at risk for development of toxoplasmosis, and the absence of antibody in patients with AIDS who have intracerebral mass lesions is strong evidence against the diagnosis of toxoplasmic encephalitis. Thus, the serologic status should be determined in all HIV-infected patients at the beginning of their infection, when they are asymptomatic, as well as in those with ARC and AIDS.

Cryptococcal infection, usually presenting with headache and fever, occurs in up to 11% of patients with AIDS, usually in the form of cryptococcal meningitis.[36] Cerebrospinal fluid pleocytosis may be minimal in patients with AIDS and cryptococcal meningitis; 69% of patients may have less than 20 leukocytes per cubic millimeter. Cerebrospinal fluid should be examined in the laboratory for cryptococcal antigen (positive in 100%), India ink stain (positive in 82%), and fungal culture (positive in 100%).[39] Isolation of *C neoformans* from CSF, blood (Isolator is the optimal method), and other specimens is essential for definitive diagnosis. The latex agglutination test for detection of cryptococcal polysaccharide antigen is a very sensitive, rapid test that can be performed on specimens of serum, CSF, urine, and body fluids. A positive titer indicates the presence of disease activity, even in patients who are minimally symptomatic. However, a prozone reaction has been observed to produce falsely negative results at low dilutions. Cerebrospinal fluid antigen titers usually fall with therapy; however, CSF titers persisting above 1:8 have been associated with relapse. Titers greater than 1:1000 are associated with a poor outcome.[40] If an intracerebral cryptococcoma is present with no associated meningitis, the cryptococcal antigen, India ink stain, and fungus culture will probably be negative.

Mycobacterium tuberculosis involvement of the central nervous system has been described in patients with AIDS and ARC who are intravenous drug abusers or Haitians.[36] Patients may present with meningitis or focal cerebral lesions (tuberculomas). The latter have been reported in a series of eight intravenous drug users with ARC.[41] The diagnosis was confirmed only by brain biopsy. Central nervous system infections with *M avium–intracellulare* are rare.

Bacterial infections of the central nervous system are rare in AIDS. Several cases of *Listeria monocytogenes* meningitis (one involving a brain abscess) have been reported.[36] Neurosyphilis was described in 1 of 186 patients with HIV infection in a Johns Hopkins series.[42]

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Biopsy Diagnosis in Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

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• Human immunodeficiency virus infection and the complications of the acquired immunodeficiency syndrome produce a broad spectrum of lesions affecting all organs and tissues. They are caused by uncommon etiologic agents and characterized by unusual location, multiplicity, rapid progression, and tendency to generalization. Cellular reactions are inefficient and histologic appearances frequently atypical. Tissue biopsy, most often endoscopic, with microscopic examination, is the method predominantly used for achieving the required rapid, definitive diagnosis. This article reviews the lesions commonly seen in biopsy specimens of human immunodeficiency virus infection and acquired immunodeficiency disease syndrome, their characteristic histologic features, and their differential diagnosis.

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The first cases of unusual opportunistic infections in homosexual men were reported by the Centers for Disease Control, Atlanta, Ga, in June 1981. In the 9 years since then, we have learned that an infection with a newly recognized retrovirus is able to undermine irreversibly the cellular immune system, causing a severe syndrome of immune deficiency that leaves the organism unprotected against a host of infectious and oncogenic agents. Most of these pathogens, although common in the environment, are rarely the cause of disease in the immune-competent general population. In the immune-deficient individuals, however, the opportunistic microorganisms produce diseases characterized by severe symptoms, high aggressiveness, tendency to generalization, resistance to treatment, and short survival. The pathology of diseases associated with the acquired immunodeficiency syndrome (AIDS) represent a broad spectrum of lesions affecting all tissues and organs. The great variety of uncommon clinical manifestations is well documented in the recent medical literature, and the virtually new field of pathology of human immunodeficiency virus (HIV) infection and AIDS has been extensively described and illustrated.[1-5] The knowledge of it is indispensable for the identification and evaluation of pathologic changes associated with the HIV infection and with the complications of AIDS.

The impact of the AIDS epidemic on practicing pathologists has been considerable both in the volume and in the novelty of pathologic material. In keeping with the theme of this conference, in this article the AIDS-associated lesions will be considered from a diagnostic point of view. Therefore, the pathologic changes will be reviewed not by disease entities but by the organ systems affected. The biopsy material of various organs as generally obtained in the laboratory will be considered, the diagnostic techniques will be indicated, and the differential diagnosis will be discussed.

LYMPH NODE BIOPSIES

The lymph nodes are affected by the initial HIV infection as well as by most of the later opportunistic complications of AIDS. Because of this unique situation, lymph nodes are one of the organs most commonly subjected to biopsy in the population at risk for AIDS. In the course of progressive generalized lymphadenopathy, biopsy of enlarged lymph nodes is performed to confirm the diagnosis of HIV infection, to obtain prognostic indications, and to rule out the presence of lymphoma. In the course of AIDS, a lymph node biopsy may elucidate the cause of a lymphadenopathy or provide the etiologic diagnosis of a systemic disease.

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The lymph node infection with HIV resulting in clinical progressive generalized lymphadenopathy is expressed by characteristic, although nonspecific, histologic patterns.[6-8] Three major histologic patterns have been described, and correlations with the progression of immune deficiency and the clinical course have been established.[3,5,9]

Pattern A generally shows features consistent with an acute lymphadenitis of viral origin (Figs 1 and 2). These features include enlarged, coalescing lymphoid follicles taking serpiginous or hourglass shapes. The follicles include markedly hyperplastic germinal centers with extensive cytolysis, phagocytosis of nuclear debris by tingible body histiocytes, and cellular regeneration with numerous cells in mitosis. The dendritic cell network is disrupted, and small, mantle lymphocytes may penetrate into the follicles. There are multiple focal hemorrhages and aggregates of transformed monocytoid B lymphocytes located next to blood vessels and sinuses. There are also clusters of polymorphonuclear neutrophils within the sinuses or associated with the monocytoid cells. Finally, there are scattered multinucleated giant cells of Warthin-Finkeldy type characterized by a grapelike cluster of overlapping nuclei.

Pattern B is intermediate between patterns A and C and includes features of both. It is characterized by the effacement of follicles and the partial involution of germinal centers. There is depletion of lymphocytes, accumulation of plasma cells, and excessive proliferation of blood vessels in and around the lymphoid follicles. These features are suggestive of subacute lymphadenitis or an intermediate phase in the progression of the HIV infection.

Pattern C shows lymph nodes with atrophic, burned-out follicles and extensive, diffuse vascular proliferation (Fig 3). The follicles are small and depleted of lymphocytes, often with a central, penetrating arteriole, surrounded by deposits of periodic acid–Schiff–positive material. In later stages, the follicles are inconspicuous and focally hyalinized. The interfollicular cortex shows marked loss of lymphocytes and excessive vascularization. There are also frequent plasma cells and diffuse fibrosis. The appearance is that of chronic lymphadenitis characterized by follicular atrophy, extensive angiogenesis, and fibrosis as previously described.[3,5,7,9] The course of progressive generalized lymphadenopathy is variable and clinically unpredictable. Presently, the most reliable prognostic indicator in HIV-infected patients is the total amount of T-helper cells and the T-helper/T-suppressor cell ratio. Recent studies have shown that additional prognostic indications can be obtained from the histologic findings of lymph nodes of progressive generalized lymphadenopathy, which show a remarkably good correlation with the general course of the disease.[5-9] Pattern A has the best prognosis, with patients being in a stationary phase sometimes for a long time. Pattern C, in contrast, is associated with the worst prognosis. Patients with the C pattern of lymph node lesions have the shortest intervals of progression to AIDS, the most severe and least curable complications, and generally the shortest survivals.[9] Pattern B seems to represent an intermediate, possibly transitional stage of progression. The pathogenesis of these lymph node lesions is not well understood. The major changes observed in pattern C, ie, the involution of lymphoid follicles with the fibrosis of the germinal centers and the excessive vascular proliferation, are similar to those seen in other types of immune deficiencies and also resemble the characteristic aspects of angiofollicular hyperplasia or Castleman's disease. A special aspect of the involvement of lymphoid tissues in the HIV infection is the salivary gland lymphadenopathy.[10] Several lymph nodes, normally embedded in the parotid gland, may become the primary site of the benign lymphadenopathy and of the malignant lymphomas associated with the HIV infection. In such cases, the greatly enlarged (often bilaterally) parotid glands show lymph node lesions

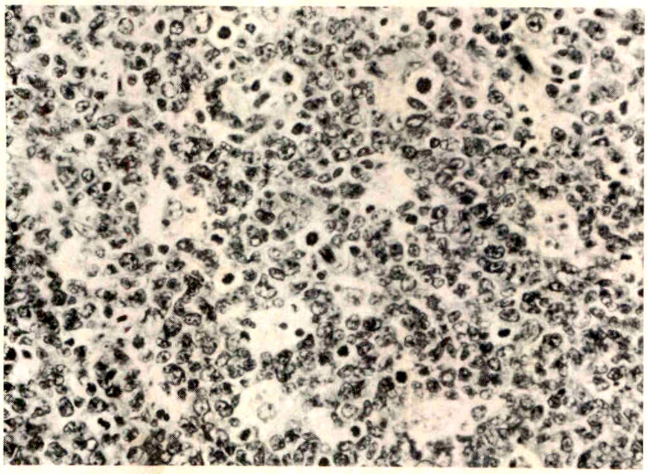

Fig 1.—Acute lymphadenitis (pattern A) of human immunodeficiency virus infection. Reactive germinal center with tingible-body macrophages containing nuclear debris, activated lymphocytes, and numerous mitoses (hematoxylin-phloxin-saffranine, original magnification ×400).

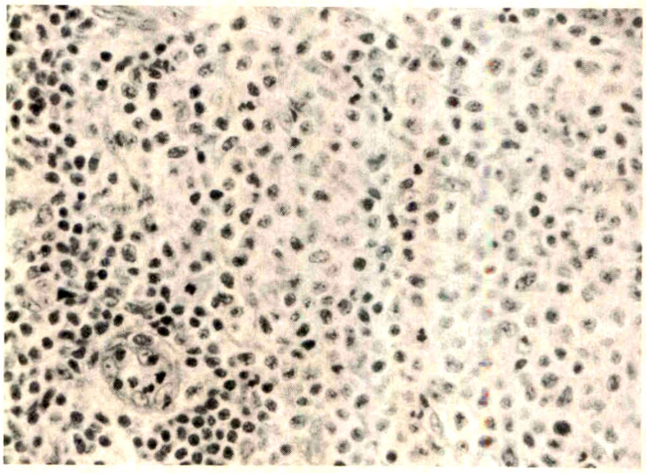

Fig 2.—Lymphadenitis (pattern A) of human immunodeficiency virus infection. Aggregate of monocytoid B cells characterized by sharp borders, clear cytoplasm, and central nuclei, with scattered granulocytes, adjacent to a hyperplastic capillary (hematoxylin-phloxin-saffranine, original magnification ×400).

similar to those present in lymph nodes at other locations as well as squamous hyperplasia and metaplasia of salivary ducts with formation of large epithelial islands and keratin cysts (Fig 4).

In the AIDS phase of the disease, lymph nodes are commonly involved in the course of infections and neoplasias. As such, they are frequently subjected to biopsy in the search for diagnosis. Mycobacterial infections, more often with the *Mycobacterium avium-intracellulare* complex than with *Mycobacterium tuberculosis*, may totally alter the lymph node structure. The cortex and parts of the medulla are totally replaced by sheets of histiocytes distended by masses of engulfed acid-fast staining bacilli (Fig 5). Necrosis, fibrosis, and calcification, hallmarks of *M tuberculosis* infection, are absent in the involvement by *M avium-intracellulare*. A special histologic form that raises an alternate set of differential diagnoses is the histoid type of lymph node mycobacteriosis. In this, the histiocytes are spindle shaped and aggregated in bundles and whorls resembling a connective-tissue tumor.[3] Like the conventional histiocytes, the spindle-shaped variants are filled with *M avium-intracellulare* bacilli that stain strongly with acid-fast bacilli, periodic acid–Schiff, and Gomori's methenamine silver stains. Among the fungi, *Cryptococcus neoformans* is the most common infectant of lymph nodes. Large areas of parenchyma are entirely replaced by masses of budding yeasts with a thick mucicarmine-positive capsule, histiocytes, and abundant mucinous substance (Fig 6). Coccidioidomycosis may also affect lymph nodes, resulting in a granulomatous reaction composed of multinucleated giant cells, epithelioid cells, and lymphocytes that is organized around the thickly capsulated sporangia containing multiple endospores. Toxoplasmosis of lymph nodes is uncommon. Usually the lymphoid response to the systemic infection with this parasite is the formation of epithelioid cells clustering in and around the lymphoid follicles. Occasionally, in AIDS, the etiologic agent is present in the lymph nodes in the form of aggregates of tachyzoites surrounded by epithelioid and giant cells.

The common neoplasms of AIDS, Kaposi's sarcoma and non-Hodgkin's lymphoma, frequently affect the lymph nodes, primarily as single locations of the tumors or secondary to a primary site in a different organ. Kaposi's sarcoma in lymph nodes replaces the lymphoid tissues with spindle-shaped, fibroblastlike cells proliferating in solid bundles or lining cleftlike rudimentary vascular spaces containing abundant red blood cells. The tumor cells have large nuclei with pointed ends, conspicuous nucleoli, and occasional mitoses. Cell staining with factor VIII antibody is variable. Among malignant lymphomas, Hodgkin's lymphomas are infrequent while non-Hodgkin's lymphomas are common, which is unlike the relative incidence of these neoplasias in the general population.[11] Also differing from the findings in patients without AIDS, lymphomas associated with AIDS are more likely to be primarily located in organs other than lymph nodes.[12-16] Although less commonly the primary site of lymphomas, the lymph nodes are involved frequently enough to justify their biopsy for the diagnosis of this malignant neoplasm. Both Hodgkin's and non-Hodgkin's lymphomas in AIDS are in most cases of high histologic grades, with a mixed cellularity type in the former and Burkitt's and immunoblastic types in the latter.[13,16] At low power, the characteristic starry-sky pattern due to the active cell lysis and phagocytosis is present in both the Burkitt's and Burkitt's-like lymphomas, the two types differing mainly by the smaller or larger size of their noncleaved nuclei. The immunoblastic lymphomas are recognized by their large, sometimes pleomorphic nuclei, single prominent nucleoli, numerous mitoses, and frequent areas of necrosis.[13,16]

BONE MARROW BIOPSIES

Persons infected with HIV may present with fever of unknown origin, fa-

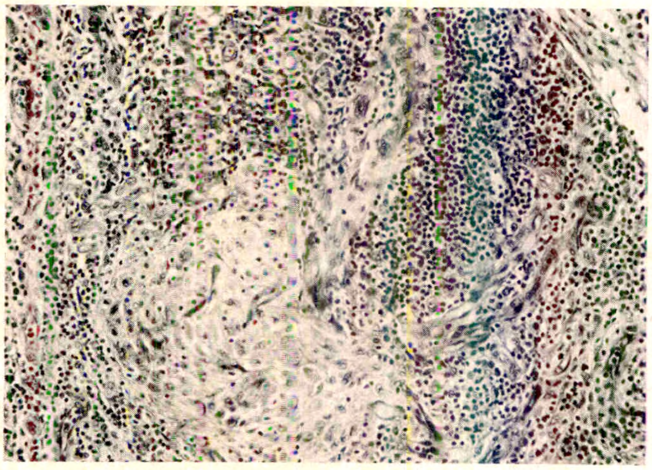

Fig 3.—Chronic lymphadenitis (pattern C) of human immunodeficiency virus infection. Burned-out lymphoid follicle with fibrosed germinal center penetrated by two arterioles from the right upper subcapsular area (hematoxylin-phloxin-saffranine, original magnification ×200).

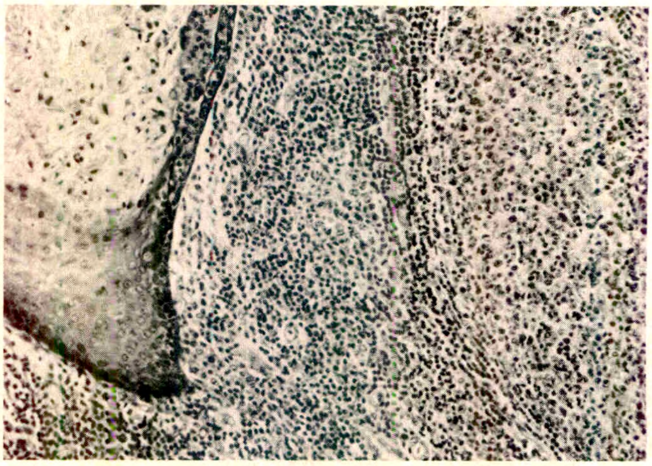

Fig 4.—Salivary gland lymphadenopathy of human immunodeficiency virus infection. Squamous cell island with keratin cyst surrounded by lymphoid tissue with hyperplastic germinal center (hematoxylin-phloxin-saffranine, original magnification ×200).

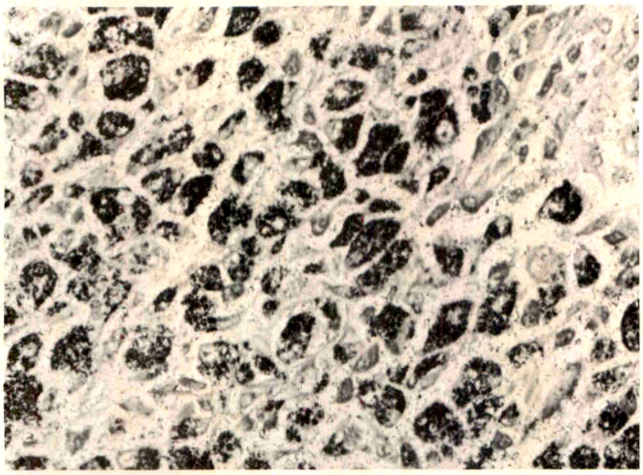

Fig 5.—Mycobacteriosis of a cervical lymph node. Histiocytes filled with *Mycobacterium avium-intracellulare* bacilli entirely replace the lymphoid cells of nodal parenchyma (Ziehl-Neelsen, original magnification × 400).

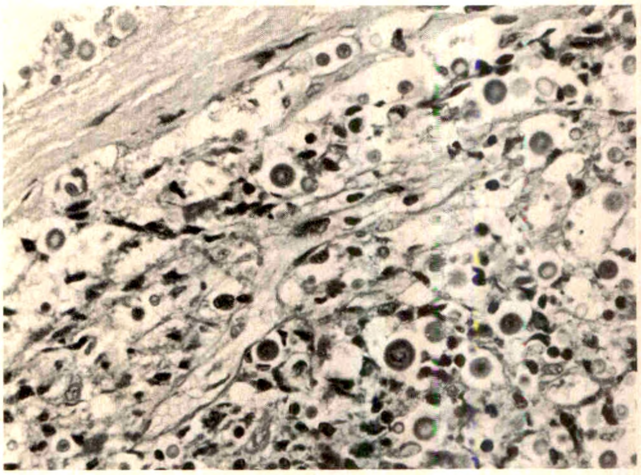

Fig 6.—Cryptococcal lymphadenitis. Subcapsular area depleted of lymphoid cells with large amounts of mucicarmine-positive yeasts (mucicarmine, original magnification × 400).

tigue, and weight loss associated with anemia, granulocytopenia, and thrombocytopenia of various degrees. To evaluate these commonly occurring forms of pancytopenia in individuals at risk for AIDS, bone marrow aspirations and biopsies are frequently performed. In patients with manifestations of AIDS, the various systemic diseases may involve the bone marrow, which then shows the local lesions of the general infection or neoplasia.[3] Granulomas with or without necrosis may be present in the bone marrow in the course of mycobacterial or fungal infections. Acid-fast and silver stains must be routinely performed to reveal the presence of *M tuberculosis*, *M avium-intracellulare*, *Histoplasma*, *Toxoplasma*, or other microorganisms. Not infrequently, however, the immunodeficient patient is unable to form granulomas, and *M avium-intracellulare* is cultured from the peripheral blood or bone marrow, while the tissue sections show no characteristic changes. In other cases, granulomas are present but acid-fast organisms cannot be demonstrated. In general, bone marrow granulomas in AIDS-associated cases are ill-formed, frequently consisting of a loose aggregate of epithelioid cells without giant cells or necrosis. Overall, culture of bone-marrow aspirates appears to be a more sensitive method than acid-fast stainings of tissues for demonstration of *M avium-intracellulare* infection.[17] Hodgkin's and non-Hodgkin's lymphomas may involve the bone marrow in patients with AIDS, often representing the primary location of the neoplastic process. Kaposi's sarcoma has not been reported to involve the bone marrow, even when it is widely disseminated.[17]

Far more common and difficult to evaluate than the specific lesions of AIDS are a number of changes in the hematopoietic bone marrow that, although nonspecific, with a few minor variations form a relatively characteristic picture.[3] In HIV-infected patients, before the debilitating complications of AIDS occur, the hematopoietic bone marrow is almost always hypercellular, in contrast to the decrease in the peripheral blood counts. Except in the late stages of AIDS, when the exhausted bone marrow finally becomes hypocellular, there is marked hematopoietic hyperplasia. The hematopoietic bone marrow is evenly expanded, separating without effacing the adipose tissue (Fig 7). The myeloid-erythroid ratio is increased, because the cellular hyperplasia affects the white blood cell series more than the red. There is also a marked increase in the total amount of megakaryocytes, which are usually present in clusters, a tendency often associated with myeloproliferative disorders. The occurrence of pancytopenia in conjunction with bone-marrow hypercellularity is an indication of ineffective hematopoiesis or of peripheral cellular destruction.

In addition to the quantitative changes, morphologic cellular abnormalities are usually noted. In the myeloid series, immature, blastlike cells with high nuclear-cytoplasmic ratios and unusual irregular, folded nuclei have occasionally been described; they are considered to represent cellular dysplasia. More striking are the morphologic changes found in the megakaryocytic series, in which abnormal, dysplastic megakaryocytopoiesis is expressed by the presence of immature and atypical forms. There are numerous megakaryocytes with hyperchromatic, poorly lobulated, apparently pyknotic nuclei surrounded by a small amount of cytoplasm; there are megakaryocytes with large, bizarre nuclei that resemble megakaryoblasts or leukemic cells; and there are bare nuclei, devoid of cytoplasm, that are scattered throughout the hematopoietic bone marrow[3] (Fig 7). Lymphocytic nodules and mixed collections of small and large lymphocytes, usually not in a paratrabecular position, are common. Increased numbers of eosinophils and markedly increased numbers of plasma cells have been recorded. These can be correlated with the presence of hypergammaglobulinemia in 83% of patients. Proliferation of reticulin fibers in various amounts is consistently observed, and excessive proliferation of blood vessels has been recorded as well. Iron stores are increased, serum iron level is low, and ferritin levels are increased. Focal deposits of hemosiderin can frequently be seen, indicating the occurrence of hemolysis.

The cellular maturational abnormalities and morphologic changes resemble those of myelodysplasia, a syndrome that is considered to represent a preleukemic state; however, flow cytometric examination has not revealed the presence of aneuploid clones.[18] The pathogenesis of bone marrow abnormalities is not well understood. These nonspecific changes often occur in the absence of any opportunistic infections in patients who were not under any drug treatment. It appears, therefore, although direct evidence is not yet available, that the peripheral pancytopenia and hematopoietic bone marrow dysplasia are direct effects of the HIV infection.

LUNG BIOPSIES

The respiratory system is a common site for serious AIDS complications, of which *Pneumocystis carinii* pneumonia affects as many as 65% of all patients with AIDS.[19,20] This situation creates the urgency of treating such severe diseases and therefore the need for rapid and precise diagnosis. Fiberoptic bronchoscopy with the use of a variety of devices and techniques is able to reach out to the periphery of the lung. Washings, brushings, and, more recently, bronchoalveolar lavage are used for the detection of *P carinii* organisms. However, a better yield of up to 95% to 98% of positive diagnosis is obtained with the transbronchial forceps biopsy.[21,22] Multiple bites produce several small tissue fragments that, before fixation, are used to make touch preparations. In our laboratory, these imprints are stained with methenamine silver by the Gomori procedure in the microwave oven, providing a rush specimen ready for examination and reporting of a preliminary diagnosis within 15 to 20 minutes. The definitive diagnosis is made on the paraffin-embedded section, where the presence of *P carinii* organisms stained with Gomori's methenamine silver can be correlated with the histologic changes as seen on the regularly stained slides. An additional section stained for acid-fast bacilli accompanies the first two slides, since the identification of one infectious agent in the pulmonary lesions of AIDS does not preclude a concomitant infection with another. *Pneumocystis carinii* pneumonia may coexist with other pulmonary infections, most commonly cytomegalovirus (CMV) and *M avium-intracellulare*, in up to 32% of cases.[19] *Pneumocystis carinii* pneumonia is expressed as a patchy involvement of alveoli with little or no participation of the interstitium. The involved alveoli are distended by a fibrinous exudate characterized by its foaminess and poor cellularity. It is strongly eosinophilic and stains well with the periodic acid–Schiff stain (Fig 8). Clusters of 4- to 6-μm ovoid cysts with one or two dark points are present within the alveolar exudates. After specific treatment, the cysts may appear fragmented and palely stained and thus more difficult to recognize. Because of the thick exudate in which they are embedded and their adherence to alveolar walls, *P carinii* cysts do not readily appear in the sputum, making its examination a less productive method for diagnosis. Transbronchial needle aspiration more recently adapted to the fiberoptic bronchoscope, which reaches further and causes less bleeding, may prove advantageous in the biopsy of AIDS-associated pulmonary lesions.[23]

The histologic presentation of *P carinii* pneumonia may include atypical features in 69% of cases or may be predominantly atypical in 30% of cases.[24] The histologic findings are considered atypical when the foamy exudate is absent, when there is considerable desquamation of alveolar epithelium, or when marked interstitial inflammation is present. Formation of epithelioid and giant cell granuloma with centers of necrosis, fibrin exudate, and *P carinii* cysts is also considered to be atypical[3] (Fig 8). The use of specific monoclonal antibodies may improve the detection of *P carinii* in the sputum and its identifi-

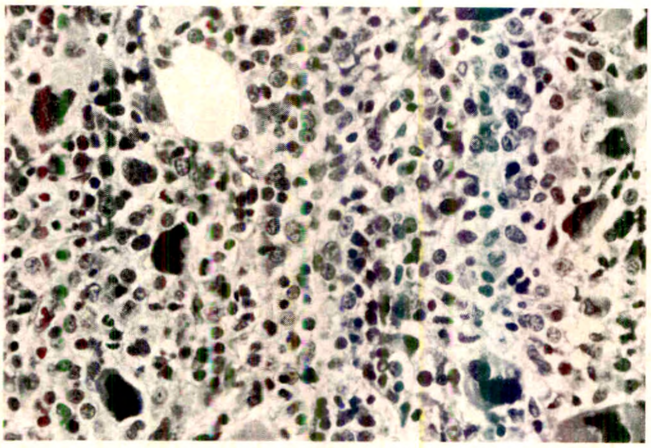

Fig 7.—Bone marrow in human immunodeficiency virus infection. Hypercellular hematopoietic bone marrow with myeloid and megakaryocytic hyperplasia. Bizarre and pyknotic megakaryocytic nuclei are frequently devoid of cytoplasm (hematoxylin-phloxin-saffranine, original magnification ×400).

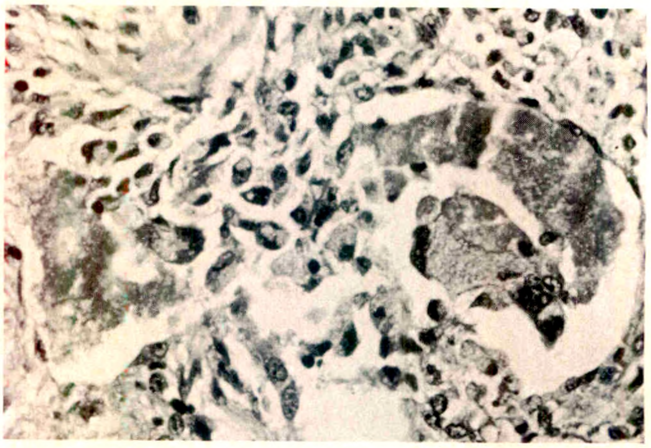

Fig 8.—*Pneumocystis carinii* pneumonia. Foamy, fibrinous, periodic acid–Schiff–positive exudate within two adjacent alveoli is partially surrounded by foreign-body giant cells (periodic acid–Schiff, original magnification ×400).

cation in tissue sections.[25]

The next most common cause of pneumonia in patients with AIDS is CMV, found in 17% of AIDS-associated pulmonary complications.[19,26-28] The effectiveness of bronchoscopy in the diagnosis of CMV pneumonia is much lower than for *P carinii*, yielding a positive diagnosis in only 55% of autopsy-proved cases.[22] Typical large, haloed, strongly basophilic intranuclear viral inclusions or multiple, small, indistinct intracytoplasmic inclusions within greatly enlarged cells can be found in the lung biopsy specimen (Fig 9). The affected cells are type II alveolar pneumocytes, macrophages, and endothelial cells, while the tissue changes resemble diffuse alveolar damage or chronic interstitial pneumonitis.[26-28] Cytomegalovirus inclusions can also be identified by monoclonal antibodies and peroxidase staining or by in situ hybridization.[3] These more laborious and costly methods are usually unnecessary given the easily identifiable features of CMV.

Mycobacterial infections are the third most common cause of pneumonitis associated with AIDS.[19,29] Both *M avium-intracellulare* and *M tuberculosis* rarely form granulomatous structures, and therefore an acid-fast bacilli stain should be routinely included in the processing of lung biopsy specimens. Aggregates of large, flat histiocytes with cytoplasmic striations are the hallmark of *M avium-intracellulare* infection, although occasionally the histiocytes may become spindle-shaped and associated in bundles and whorls similar to the appearance of histoid leprosy.[3] In the presence of a solid tumorlike area composed of spindle-shaped cells, the possibility of *M avium-intracellulare* infection must therefore be considered, along with those of Kaposi's sarcoma and sarcomatous neoplasms. The distinction, however, is simple with the acid-fast bacilli stain because *M avium-intracellulare* bacilli, when present, are in large amounts, filling the histiocytes and causing their striated appearance. This is in sharp contrast to the *M tuberculosis* infection, in which, even in the presence of extensive, often caseating lesions, very few bacilli are present, requiring a long and careful search for their finding. Although the *M avium-intracellulare* infection is still predominant, *M tuberculosis* infections are gradually increasing as the risk of AIDS shifts more from homosexual to drug-abusing patients.

The fungal infections of the lung in AIDS include cryptococcosis, histoplasmosis, coccidioidomycosis, candidiasis, aspergillosis, blastomycosis, and mucormycosis.[30] Of these, cryptococcosis is the only major consideration, particularly in urban locations.[31] Histoplasmosis and coccidioidomycosis represent a serious threat in the areas where they are endemic, the central and southwest United States, respectively, although sporadic cases, particularly of the former, may occur in other places.[32] Candidiasis, although the most common fungal infection in AIDS, is rarely a cause of pneumonia, while pulmonary infections with *Aspergillus*, *Mucor*, and *Blastomyces*, although unusual, represent severe life-threatening complications. On the lung biopsy specimen, cryptococcosis may be seen as nodules composed of scattered or confluent noncaseous granulomas, exudative pneumonia, or interstitial pneumonitis.[3,31] The fungus is monomorphic, forming yeasts but not hyphae, 4 to 15 μm in diameter with a thick mucopolysaccharide capsule that appears as a clear space around the yeast in the hematoxylin-eosin stain or strongly stained in the periodic acid–Schiff or mucicarmine stains. Budding is more common in cryptococcosis than in other yeasts, with single or double narrow-based buds that detach easily, reproducing the original cell. In histoplasmosis, the yeasts are inside macrophages where they multiply, taking advantage of the cells' loss of fungicidal capacity. The parasitized histiocytes form sheets of cells that replace normal tissues and often undergo central necrosis. The differential diagnosis of *Histoplasma* in the tissue sections includes *Cryptococcus*, with its distinctive thick capsule; coccidioidmycosis, which consists of larger, double-contoured spherules filled with endospores; and *Toxoplas-*

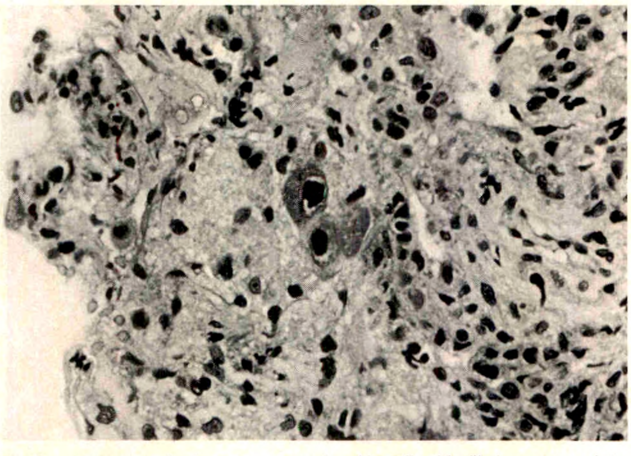

Fig 9.—Cytomegalovirus pneumonia. Alveoli with fibrinous exudate and two enlarged alveolar cells with intranuclear cytomegalovirus inclusion bodies (hematoxylin-phloxin-saffranine, original magnification ×400).

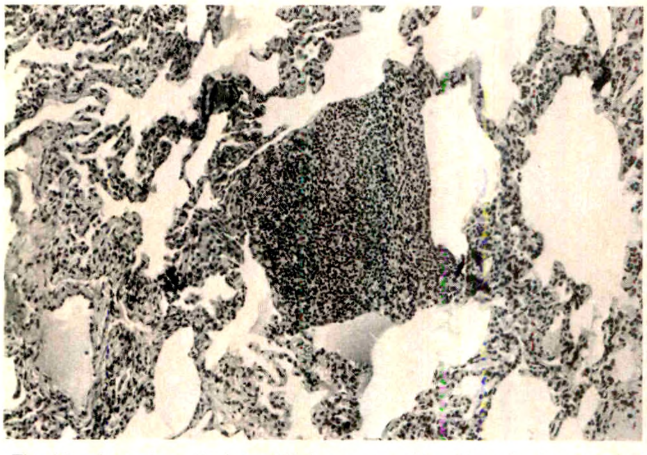

Fig 10.—Lymphocytic interstitial pneumonitis. Sharply demarcated lymphocytic nodule in interalveolar septum, with alveoli uninvolved (hematoxylin-phloxin-saffranine, original magnification ×100).

ma, which is slightly smaller, not intracellular, and not stainable by silver. Finally, aspergillosis is characterized by septate, dichotomic hyphae with a tropism for vascular lumina, which they occlude, causing areas of pulmonary infarction.

Pulmonary involvement in the form of lymphocytic interstitial pneumonitis not associated with any of the common infections of AIDS is relatively frequent, particularly in children, where in particular populations it may be present in up to 43% of cases. The diagnosis is made histologically through examination of lung tissues obtained by transbronchial biopsy. The lesions appear as randomly distributed interstitial infiltrates of lymphocytes, plasma cells, and immunoblasts with formation of nodular aggregates around the vessels and bronchioles (Fig 10). No infectious agents have been identified; however, the role of HIV is suggested by positive findings with in situ hybridization and anti-HIV antibodies in the bronchoalveolar lavage fluids. The activity of HIV is also suspected in a number of cases in which progressive pulmonary hypertension associated with pulmonary plexogenic arteriopathies occurred in HIV-infected patients.

The neoplasias of AIDS, Kaposi's sarcoma, and non-Hodgkin's lymphoma involve the lungs, more often secondarily; however, the possibility of their occurrence must be kept in mind when examining a pulmonary biopsy specimen, particularly since they may occur in association with various infections and even together, intimately admixed (Fig 11).

Kaposi's sarcoma in the lung may appear as tracheobronchial mucosal patches and plaques, as discrete peribronchial or perivascular small nodules with a lymphatic distribution, or as solid sheets distending the interalveolar septae and compressing without destroying the alveolar spaces.[33,34] As in other locations, the tumor masses consist of the characteristic spindle cells with occasional mitoses lining congested cleftlike vascular spaces. Plasma cells are usually associated with the neoplastic process. A transbronchial biopsy may not be adequate for a firm diagnosis, in which case an open lung biopsy should be requested. Non-Hodgkin's lymphomas are more often extranodal in patients with AIDS than in the general population; however, the lung is not often the primary site. Nevertheless, the pulmonary involvement must be diagnosed, and therefore any atypical lymphoid infiltration in a lung biopsy specimen has to be carefully analyzed. As in other locations, the lymphomas are of high grade, mostly Burkitt's or immunoblastic types. If frozen tissue is not available for immunoglobulin restriction identification, stainings for B- and T-cell phenotypes on paraffin-embedded tissues are still helpful by demonstrating the cell origin of the majority of infiltrating lymphoid cells.[3]

DIGESTIVE TRACT BIOPSIES

Mouth

The tongue, gums, tonsils, and remaining oral mucosa may be affected by candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, and non-Hodgkin's lymphoma, for the diagnosis of which a biopsy may be necessary. *Candida albicans*, often appearing as confluent, thick white patches on the tongue, shows colonies of yeasts and pseudohyphae on histologic examination. Oral hairy leukoplakia or condyloma planum, also localized predominantly on the tongue, particularly on the lateral borders, consists of hairlike, firmly adherent, whitish excrescences.[35] Histologically, hairy projections of keratin are seen on the surface of the squamous epithelium, which also shows acanthosis, parakeratosis, koilocytotic atypia, and focal ballooning of squamous cells. Thus, the microscopic picture resembles that of flat condylomas commonly seen in the anogenital areas. Human papillomavirus, considered the cause of anogenital condylomas, is also present in condyloma planum of the mouth, along with Epstein-Barr virus, a virus also associated with dysplasia and neoplasia.[36] Kaposi's sarcoma with the same histologic appearance as in other locations is often seen in the mouth in the form of red-purple patches and plaques on the hard palate above the level of the second molar, gums, tongue, and tonsils. Non-Hodgkin's lymphoma may localize and even originate in the gums and the salivary gland lymph nodes.[3,10]

Esophagus

Candidiasis of the esophagus occurs often and is included in the definition of AIDS. Gray pseudomembranes covering irregularly shaped ulcers appear microscopically as fibrin and necrotic tissue debris within meshworks of pseudohyphae. When yeasts predominate, the lesions tend to be shallow, in contrast to the pseudophyphae, which are the aggressive form and may extend deep into the muscular wall of the esophagus.[24] Herpes simplex virus 1 producing lesions above the diaphragm is the common cause of esophageal ulcers in AIDS. The distal esophagus is more often affected by punched-out ulcers with raised granular margins that tend to enlarge and to become superinfected with *Candida*. The typical lesions are seen in the margins of the ulcer and consist of squamous cells with ballooning degeneration and multinucleated giant cells with nuclear molding and intranuclear inclusions. The latter are lilac stained with a ground-glass appearance filling the nucleus entirely in an early phase and strongly eosinophilic with a characteristic halo at a later stage. The underlying stroma is congested and infiltrated by granulocytes. Kaposi's sarcoma may also affect the esophagus.

Stomach

Herpetic ulcers, patches of Kaposi's sarcoma, and infiltrates of non-Hodgkin's lymphoma may be observed in the stomach, although less frequently than in other segments of the digestive tract.

Small and Large Intestine

A large proportion of patients with AIDS first see a physician for gastrointestinal tract symptoms, particularly diarrhea. For this reason, endoscopic biopsy is a common procedure, often determining the etiologic diagnosis. The most frequent intestinal infection in AIDS is that with CMV, which can be easily diagnosed in the endoscopic biopsy specimen by recognition of the characteristic intranuclear inclusions. Within the gastrointestinal tract, the colon shows the largest number of CMV inclusions and the ascending colon more than the descending colon or the rectum.[37] The involvement of the colon is usually patchy, and therefore multiple colonoscopic biopsies and multiple-level sections are recommended. Single or multiple ulcers, usually well circumscribed and shallow, are present in more than 50% of cases.[37] Endothelial cells, macrophages, and smooth-muscle cells as well as colonic crypt epithelia are the sites of inclusions. As in other organs, the CMV-infected cells may appear as nuclear amphophilic inclusions surrounded by a clear halo with the typical owl-eye appearance, as cytoplasmic granular amphophilic or eosinophilic inclusions, or as markedly enlarged cells without clearly defined inclusions[24] (Fig 12). *Candida, Shigella, Mycobacterium,* and *Cryptosporidium* may be associated with the CMV infection. The bacilli of *M avium-intracellulare* may infect the intestine, resulting in areas of thick, short villi with discoloration of the mucosa. The histologic appearance resembles that of Whipple's disease, with the mucosa infiltrated by large amounts of microorganisms.[38] *Cryptosporidium,* an animal parasite, can infect the intestine of patients with AIDS, causing severe diarrhea, malabsorption, dehydration, and electrolyte imbalance. The parasites are preferentially located in the small intestine but also in the cecum, colon, rectum, and, on occasion, gallbladder. In the intestinal biopsy specimen, the organisms are seen as oval bodies, 2 to 6 μm long, located on the brush border of the epithelial cells lining the tips and sides of the villi or the lumina of the crypts[39] (Fig 13). The crypts and villi appear normal, while the lamina propria is infiltrated by lymphocytes and plasma cells. *Cryptosporidium* stains well with Giemsa, hematoxylin-eosin, and methylene blue but not with acid-fast or Gomori's methenamine silver stains.[40] Kaposi's sarcoma may be seen in the duodenum, ileum, and colon and even more frequently in the anorectal area. Non-Hodgkin's lymphoma, usually of Burkitt's or Burkitt's-like type, can also involve the small or large intestine, sometimes as the primary site. A particular localization of AIDS-associated lymphoma is in the terminal rectum and anus.[41] The patients present with anorectal symptoms and may undergo surgery for anal fistulas. Even in the case of large tumor masses, staging may not reveal other organ involvement. Anal vesicles and ulcers may be caused by infection with herpes simplex virus or CMV showing on biopsy specimens the characteristic intranuclear inclusions of these viruses. Finally, the anal squamous epithelium in patients at risk for AIDS may show a spectrum of lesions ranging from condyloma to Bowen's disease to squamous cell carcinoma. As in the genital tract, they appear to be associated with various types of human papillomaviruses, which can be visualized by immunohistochemical staining or in situ hybridization.[42] Condyloma acuminatum, planum or endophytic, exhibits characteristic koilocytotic atypia also present in the more superficial cells of Bowen's disease and squamous cell carcinoma, thus indicating the unity of these lesions and their relationship to human papillomavirus (Fig 14). In Bowen's disease or squamous cell carcinoma in situ, the cells also show lack of orientation, strong basophilia, marked nuclear pleomorphism, including giant forms, and numerous atypical mitoses. The subjacent stroma, which is not infiltrated by the tumor cells, contains abundant lymphoplasmacytic infiltrates. Invasive carcinomas in this area may be of squamous, transitional, or basaloid type, the latter representing variants of the more malignant cloacogenic carcinoma.

Liver

Needle biopsies of the liver are often used in the workup of a febrile syndrome of unknown origin in persons with the HIV infection or AIDS. The finding of small epithelioid and giant-cell, nonnecrotizing granulomas randomly located in the hepatic parenchyma is suggestive of infection with *M tuberculosis* or *M avium-intracellulare.* The Ziehl-Neelsen stain may reveal large amounts of acid-fast bacilli

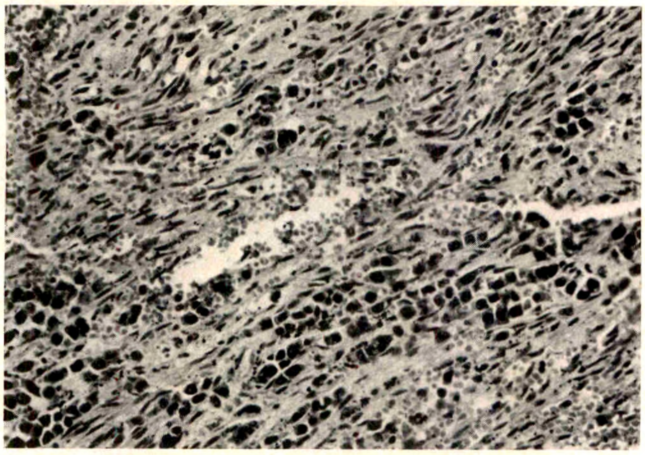

Fig 11.—Pulmonary Kaposi's sarcoma and non-Hodgkin's lymphoma. Sheets of spindle-shaped Kaposi's sarcoma cells replacing lung parenchyma and forming erythrocyte-containing slitlike spaces are infiltrated by lymphoma cells (lower right) (hematoxylin-phloxin-saffranine, original magnification ×200).

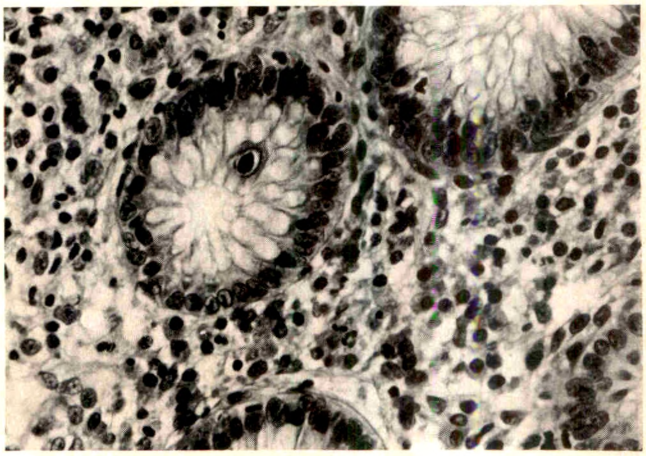

Fig 12.—Cytomegalovirus colitis. Mucosa of descending colon with an intranuclear cytomegalovirus inclusion body in a crypt goblet cell. Neutrophils, lymphocytes, and plasma cells infiltrate the lamina propria (hematoxylin-phloxin-saffranine, original magnification ×400).

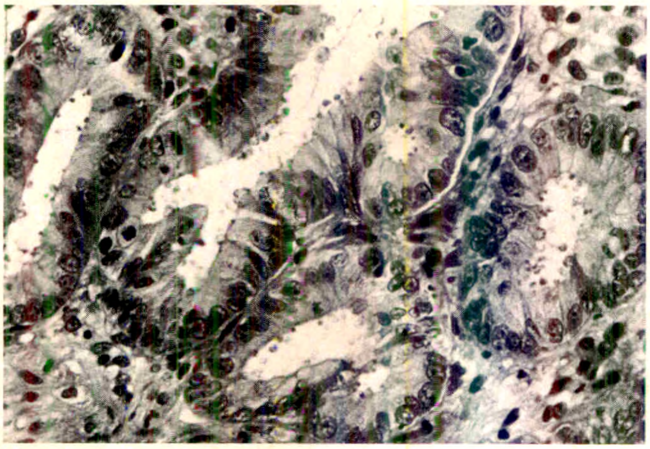

Fig 13.—Cryptosporidiosis colitis. Colonic crypts with numerous cryptosporidial sporozoites attached to the apical poles of lining epithelial cells (hematoxylin-phloxin-saffranine, original magnification ×400).

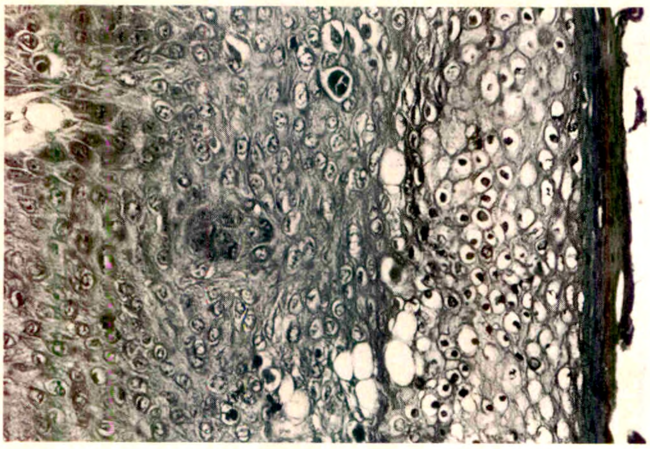

Fig 14.—Bowen's disease of the anus. Koilocytosis, parakeratosis, nuclear pleomorphism, and mitoses in markedly thickened anal squamous epithelium (hematoxylin-phloxin-saffranine, original magnification ×200).

within macrophages in the case of *M avium-intracellulare* and few or none in the case of *M tuberculosis* infection. A silver stain, which must always accompany the acid-fast bacilli stain, may reveal the presence of small, fine, budding yeasts, identified as *Histoplasma capsulatum*, as the cause of hepatic granulomas[3] (Fig 15). Chronic hepatitis is frequent, and 76% of patients in one study had serologic or pathologic evidence of hepatitis exposure.[43] However, despite the frequency of markers for hepatitis B in the AIDS population, the incidence of significant liver disease due to hepatitis B virus is small, possibly due to the background of immune suppression.[44] In addition to its use in the diagnosis of various systemic infections and in the monitoring of viral hepatitis, the liver needle biopsy in patients with AIDS may also detect the occasional presence of Kaposi's sarcoma and malignant lymphoma.

SKIN BIOPSIES

Cutaneous lesions are common in patients with AIDS and, in the majority of cases, are identifiable grossly. Confirmation by biopsy, however, is necessary in some, including herpetic ulcers, molluscum contagiosum, Kaposi's sarcoma, and lymphoma. Herpes simplex virus 1 causes lesions of the face, lips, neck, nipples, ears, and corneas, while herpes simplex virus 2 produces lesions on the perineum, external genitals, and perianal area. The vesicles that appear in 3 to 5 days from infection are followed by pustules and ulcers that are long lasting. In the biopsy specimen of a herpetic ulcer, the diagnosis is based on the presence of ballooning degeneration of the squamous epithelium, the multinucleated giant cells, and the characteristic intranuclear viral inclusions. In molluscum contagiosum, the dome-shaped umbilicated papules show the large diagnostic molluscum bodies inside markedly enlarged keratinocytes. Kaposi's sarcoma is the most common reason for a skin biopsy in patients with AIDS. The location of Kaposi's sarcoma lesions is more varied and widespread than in patients with the classic form of the disease.[3] On the face, the most common sites are the tip of the nose, the medial third of the eyelid, the earlobes, and the area behind the ears.[2,45] Other common locations are the trunk, the penis, and the dorsum of the feet. While the histologic picture is characteristic in older, nodular lesions, it is sometimes difficult to recognize in early patch lesions. These appear as a fine lacework of irregularly shaped, thin-walled capillaries with a few scattered plasma cells. The lesion is restricted to the superficial dermis, contiguous with preexisting vessels, and often resembling pyogenic granuloma.[3] Lymphomas of the skin are rare and are usually secondary to other organ involvement.

BRAIN BIOPSIES

The nervous system is commonly involved in AIDS, with 20% to 30% of patients presenting with neurologic signs and symptoms during life and 50% to 85% showing neuropathologic lesions at autopsy.[46] For this reason, biopsies of the brain are frequently performed and biopsies of peripheral nerves are occasionally performed.

The AIDS dementia complex, a form of subacute encephalitis, is the result of direct infection of the brain by HIV, which affects 40% to 60% of all patients.[47,48] The histologic lesions consist of haphazardly scattered small collections of multinucleated giant cells, macrophages, and lymphocytes within the white matter of the cortex, midbrain, brain stem, and spinal cord as well as deep in the gray matter of the thalamus and basal ganglia.[47,48] The giant cells, considered a marker for HIV infection of the brain, are located near small blood vessels and resemble the giant cells of HIV lymphadenopathy, having overlapping nuclei and very little cytoplasm.[46,49] Human immunodeficiency virus particles and genome have been detected in the giant cells of HIV encephalitis by electron microscopy and in situ hybridization.[46-49] The poorly defined collections of cells are located in areas of demyelination. Mineralization of blood vessels in the form of iron and calcium deposition in vessel walls is associated with advanced cases and is more common in children.[49]

Among the opportunistic viral infections, CMV, herpes simplex virus, and JC virus can produce a variety of neuropathologic changes. Cytomegalovirus is associated with areas of demyelination and focal necrosis. Microglial nodules and typical intranuclear viral inclusions can be seen in the hypothalamic region but also in the spinal cord and in peripheral nerves. Cytomegalovirus shows an affinity for ependymal surfaces with mononuclear cell infiltrates and intranuclear inclusion on the choroid plexus.[46] Herpetic encephalitis, far less common, will also show necrotizing lesions more often in the frontal and temporal lobes, surrounded by lymphocytic infiltration. Microglial nodules with intranuclear inclusions are also noted. The JC virus, a member of the papovavirus group, is the cause of progressive multifocal leukoencephalopathy. The definitive diagnosis can be made only by brain biopsy, in which specimens the characteristic lesions can be recognized. These consist of randomly distributed areas of demyelination with foci of infarctlike necrosis in the internal capsule. Enlarged, bizarre astrocytes, lipid-laden macrophages, and amphophilic intranuclear inclusions without surrounding haloes are present.[46,50]

Cryptococcosis is the most common fungal infection of the central nervous system in patients with AIDS. It involves mainly the meninges and the adjacent brain tissue. The meninges are thickened and opacified and the brain is covered by a cloudy, gelatinous fluid. Numerous free yeasts, lymphocytes, and plasma cells infiltrate the meninges. In chronic forms, more frequently localized at the base of the brain, the yeasts abound in the periarterial Virchow-Robin spaces, which become distended and form small cystic spaces.[24] Multinucleated histiocytic giant cells with phagocytosed yeasts are usually present. The *Cryptococcus* yeasts are easily identified by their silver staining and particularly their strong affinity for periodic acid–Schiff and mucicarmine stains. Toxoplasmosis of the brain is the most frequent parasitic infection of the central nervous system in AIDS. In the acute phase, free tachyzoites are scattered in the tissues, while they appear as cysts in the chronic phase.[51] The infection is associated with extensive tissue necrosis caused by vascular thrombosis. Tachyzoites and *Toxoplasma* antigen are predominantly localized in vessel walls, where they induce hypertrophy, inflammation, and thrombosis.[51] Early definitive diagnosis is essential for effective chemotherapy and can be improved by using immunostaining with anti-*Toxoplasma* antibody on the brain biopsy specimens. Kaposi's sarcoma, although one of the most common manifestations of AIDS, has not been reported to occur in the brain. In contrast, non-Hodgkin's lymphomas are frequently seen in AIDS, with the brain as the primary location in about 13% of all cases. This is more than 10 times the incidence in the general population.[3,46] The primary lesions are intraparenchymal and usually angiocentric, while the less frequent secondary lymphomas of the central nervous system are more likely to involve the leptomeninges with the subjacent cerebral tissues[46] (Fig 16). Their high aggressiveness is reflected in the extensive cerebral necrosis that is usually part of the lesions. The histologic features are those of high-grade lymphoma of Burkitt's or Burkitt's-like type, and the phenotype is B cell in all cases reported so far.[13,52] Peripheral nerve biopsies are occasionally performed for the various neuropathies and palsies experienced by patients with AIDS. They usually show intense inflammatory cell infiltrates and focal demyelination with accumulation of macrophages suggestive of autoimmune cellular reactions.[46]

CONCLUSION

The recognition of pathologic changes associated with HIV infection and with AIDS has progressed rapidly, unveiling a broad spectrum of lesions. The knowledge related to their etiology and pathogenesis that is essential for their understanding is also expanding at a quick pace. Translated into the everyday pathology practice, the diagnosis of HIV infection and AIDS requires the etiologic identification of a variety of patholog-

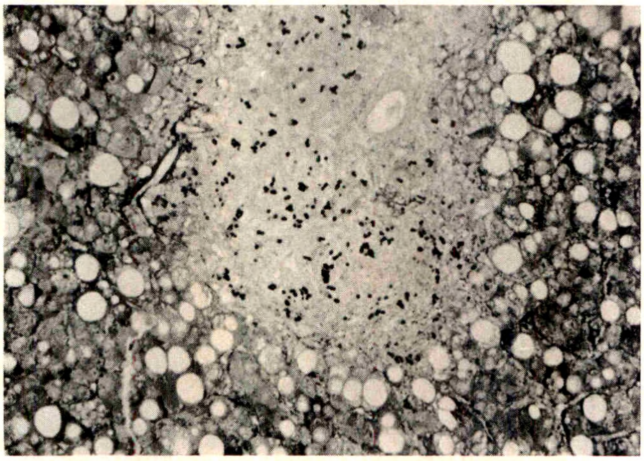

Fig 15.—Hepatic histoplasmosis. Nonnecrotizing granuloma containing silver-positive, single or budding yeasts in liver parencyma with marked fatty change (Gomori's methenamine silver, original magnification ×200).

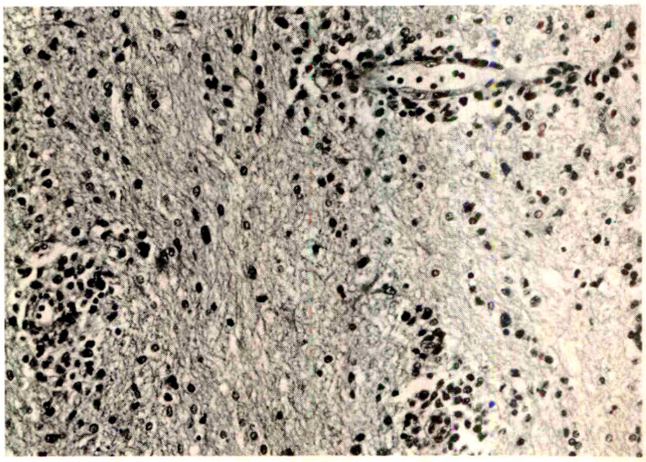

Fig 16.—Non-Hodgkin's lymphoma of the brain. Perivascular and diffuse infiltration of cerebral cortex by undifferentiated lymphoma cells (hematoxylin-phloxin-saffranine, original magnification ×200).

ic entities by the application of rapid, effective techniques and expert interpretation. The tissue lesions described in this article, in keeping with its title, refer specifically to pathologic features more likely to be seen in biopsy material. This is intended to serve as a practical guide and therefore has deliberately left out information and discussions on the mechanisms of pathologic changes.

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Transfusion of the Patient With Acquired Immunodeficiency Syndrome

Klaus Mayer, MD

• Cytopenias are commonly found in patients infected with human immunodeficiency virus. Thrombocytopenia due to peripheral destruction of platelets is an early manifestation, normocytic anemia and neutropenia are late manifestations. Transfusions are required when symptoms become severe. Chemotherapy of patients with the acquired immunodeficiency syndrome (AIDS) is also cytotoxic and further contributes to the transfusion requirement. Patients with AIDS receiving treatment with zidovudine, in particular, have a high transfusion requirement. Empirically, many centers irradiate blood products transfused to AIDS patients to prevent possible graft-vs-host reaction in the immunologically compromised recipient. Attempts to correct the deficiency of helper T cells with lymphocyte transfusion and marrow transplantation have so far been ineffective. Similarly, therapeutic trials to treat this disease by apheresis of virus-bearing cells has been ineffective and has, therefore, been abandoned.

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Nowadays transfusion-associated retroviral infection occupies a major role in the life of the blood bank director. The converse is also true because patients with the acquired immunodeficiency syndrome (AIDS) have major transfusion requirements and these needs are increasing. All circulating blood cells of the patient with AIDS may be affected. Leukopenia is common, and this is most pronounced in the finding of a low absolute lymphocyte count. This is in no way surprising, because the human immunodeficiency virus invades the helper T cell, destroys it, and thereby creates havoc by interfering with the host response to infection. The resultant opportunistic infections are usually the ultimate cause of the inevitable death of patients with AIDS. Early on lymphocyte transfusions were considered to correct this lesion.[1] Such transfusions, if successful, can lead to graft-vs-host disease. At best, the reversal of the immune deficiency is only short-lived. Although cytotoxicity of natural killer cells can be enhanced by incubation in vitro with interleukin 2, this approach also fails because no notable long-term clinical benefits result.[2,3] Such strategies continue to receive attention but, because they are believed to be ineffective, they have generally been abandoned.

Although the major clinical manifestations of AIDS are opportunistic infections and malignant neoplasms, some hematologic defects are more amenable to therapy. In a review published by Zon et al,[4] of 102 patients studied at New England Deaconess Hospital, Boston, Mass, more than half who developed the full picture of AIDS also developed anemia, leukopenia, and/or thrombocytopenia. Not surprisingly, the incidence of these blood cell deficiencies progressed with the severity of the disease, starting with asymptomatic carriers progressing to AIDS-related complex and finally AIDS and Kaposi's sarcoma.

Bone marrow studies on patients with AIDS reveal a diverse picture. The marrow is usually normocellular, but increased cellularity may also be present, and an aplastic aspirate is occasionally seen. Increase in plasma cells and rouleaux formation of red blood cells are encountered. A dysplasia of erythroid and myeloid elements is not uncommon. Typical marrow aspirates are depicted in the Figure. When marrow aspirations and biopsies are performed in patients with AIDS, the opportunity should not be lost to culture the aspirate. The findings are frequently positive, permitting an early diagnosis of one of the many opportunistic microorganisms often found to infect these patients. Frequently marrow cultures for mycobacteria are positive.

ANEMIA

Anemia is a common finding. When it is present, it is usually normocytic.

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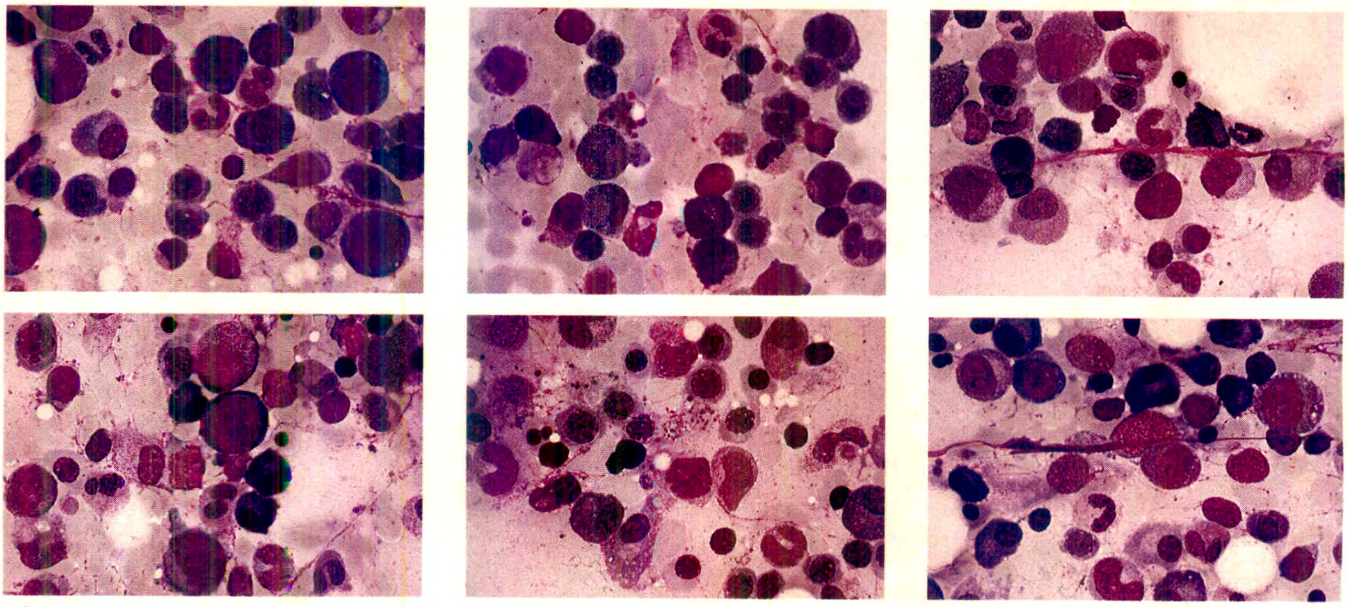

Typical marrow aspirate (Wright and Giemsa, × 1000).

Transfusions of red blood cells are occasionally required but are often unnecessary because these are chronic anemias acquired gradually. Only clinically symptomatic individuals need to be transfused. Low hemoglobin levels are tolerated well by this youthful patient population. At Memorial Sloan-Kettering Cancer Center, New York, NY, 349 of 790 patients with AIDS received 3229 U of red blood cells; therefore, an average AIDS patient with AIDS received 9 U of red blood cells.

Those patients repeatedly transfused may, of course, develop alloantibodies. The antibodies found in 630 tested patients with AIDS are given in Table 1. Ten percent of these individuals were sensitized, compared with 6% of other Memorial Hospital patients. Nineteen percent of these patients developed a positive direct antiglobulin test. The character of coating is given in Table 2.

Occasionally autoimmune hemolytic anemias due to IgG autoantibodies are encountered. More often IgM antibodies are found. This may be related to the frequent occurrence of pneumonia.

To date, the treatment most commonly tried for AIDS is zidovudine—a thymidine analog that inhibits replication of the retrovirus. A common toxic effect is the development of an anemia, usually due to reduction in erythropoiesis and marrow aplasia. Occasionally, megaloblastic changes unrelated to vitamin B_{12} or folate deficiency are noted. Erythropoietin levels are usually elevated. Trials are underway, and evidence suggests that, despite normal or elevated erythropoietin titers, erythropoietin therapy may be effective. These patients, in later stages, may require red blood cell transfusions. Again, this approach is reserved for those symptomatic with chronic anemia.[5,6]

THROMBOCYTOPENIA

Thrombocytopenia is a common finding in patients with AIDS. It is often seen early in the course of the disease before any other symptoms have become apparent. More than 40% of the patients with AIDS exhibit a thrombocytopenia.[4] Megakaryocytes are present in increased number in the bone marrow, suggesting that the mechanism of the thrombocytopenia is the peripheral destruction of platelets. Platelet-bound IgG, complement and immune complexes are substantially increased in these patients. The peripheral destruction is usually moderate, less so than in idiopathic autoimmune thrombocytopenia. Because the platelet counts are rarely low enough to cause bleeding, transfusion of platelets is required only occasionally. At Memorial Sloan-Kettering Cancer Center, 89 patients received 1437 U of platelets or a mean of 16 U per patient. In addition, as with all thrombocytopenias due to peripheral destruction of platelets, there is a skewing of the age population of platelets in favor of young, hemostatically more effective cells. Whereas a patient with a platelet count of 20×10^9/L with acute leukemia or aplastic anemia will have a grossly abnormal bleeding time, bleeding time is much shorter in patients with idiopathic thrombocytopenic purpura or thrombocytopenia secondary to AIDS.

Table 1.—Alloantibodies Detected in 630 Patients With Acquired Immunodeficiency Syndrome*

Antibody	No. of Patients
Auto Anti I	15
Anti IH	15
Anti WBC	11
Anti Le^a	7
Anti P_1	7
Anti M	2
Anti E, K, Lu^a	1
Anti E, WBC	1
Anti K	2
Anti A_1	1
Anti Sd^a	1
Total	**63**

*Ten percent of the patients tested had antibodies compared with 6% with antibodies in our overall patient population.

Because most hemophiliacs have become infected with human immunodeficiency virus-I,[7] their coagulation defect often is complicated by thrombocytope-

Table 2.—Results of Direct Antiglobulin Test in 630 Patients With Acquired Immunodeficiency Syndrome*

		Character of Coating	
No. of Patients	**Poly Specific**	**IgG**	**Complement 3**
38	+	NT	NT
57	+	+	–
3	+	+	+
16	+	–	+
7	+	–	–

*A total of 121 (19.2%) of 630 patients had a positive direct antiglobulin test. Further complement testing revealed the character of the coating. Plus indicates a positive test result; minus, negative test result; and NT, not tested.

nia. Goldsmith and Kisker[8] pointed out that these hemophiliac patients can be treated conservatively. They rarely require platelet transfusion. Corticosteroid therapy and splenectomy are immunosuppressive and should be used only in extreme circumstances in patients susceptible to opportunistic infection.[8] An approach successfully used is the transfusion of large quantities of intravenous gammaglobulin.[9]

Although neutropenia is common in the late stages of AIDS, it is almost never sufficiently acute and severe to warrant granulocyte transfusion.

GRAFT-VS-HOST REACTION

Transfusion of cellular products to immunodeficient recipients may result in engraftment. This in turn may cause severe, even fatal, graft-vs-host disease. These observations were made in patients with cancer who received large doses of cytotoxic chemotherapy and in neonates who undergo blood transfusion. Those infected with the human immunodeficiency virus are probably similarly susceptible. To date, no significant reports of this occurrence have been published. The reason may be that in many centers where these patients are treated, all blood products transfused to patients with AIDS undergo prior irradiation. Although this procedure seems logical and in itself harmless, it has not been proved necessary and may be an unnecessary expense.

CONCLUSION

Cytopenias are found commonly in patients infected with the human immunodeficiency virus. Thrombocytopenia due to peripheral destruction of platelets is an early manifestation. Normocytic anemia and neutropenia are late manifestations. Transfusions are required when symptoms become severe. Therapeutic trials may be accompanied by further marrow suppression also requiring transfusion. Empirically, many centers irradiate blood products to prevent possible graft-vs-host reaction in the immunologically compromised recipient. Attempts to correct the deficiency of helper T cells and marrow transplantation have so far been ineffective.

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The Significance of HIV-1–Indeterminate Western Blot Results in Blood Donor Populations

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• Until recently it has been difficult to counsel blood donors with human immunodeficiency virus-1 (HIV-1)–indeterminate Western blot results due to a lack of information concerning the significance of this finding. Reports from several investigators have provided laboratory, epidemiologic, and prospective follow-up data that have clarified the potential significance of this laboratory finding. It has been established that the band patterns observed in an HIV-1–indeterminate Western blot result are important in predicting the significance of the result; specifically, the absence of p24 or envelope glycoprotein reactivity has not been associated with HIV-1 infection, whereas the presence of p24 reactivity has infrequently been the first sign of HIV-1 seroconversion. These data provide the basis for formulating policies concerning the notification and counseling of blood donors with HIV-1–indeterminate Western blot patterns.

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During the initial stages of implementation of anti–human immunodeficiency virus, type 1 (anti–HIV-1) testing, the Western blot assay was regarded as the confirmatory laboratory procedure that could definitively establish whether or not a positive anti–HIV-1 enzyme immunoassay (EIA) screening test result represented true exposure to HIV-1.[1] However, it soon became apparent that there were instances where the Western blot did not yield a definitive interpretation.[1-3] Thus, the category HIV-1–indeterminate Western blot result was established to characterize specimens that showed reactivity insufficient to be interpreted as diagnostic of HIV-1 infection.

Recently, the Centers for Disease Control, Atlanta, Ga, defined revised criteria for a positive HIV-1 Western blot result.[4] These criteria state that reactivity must be present to at least two of the following three antigens: p24, gp41, and gp120/160. In the same publication, a negative Western blot result is defined as the absence of all bands. An indeterminate Western blot result has therefore been defined as any pattern that does not fit the definition of a positive or negative result.

Data accumulated over the last several years indicate that the significance of an HIV-1–indeterminate Western blot result is dependent on the observed band pattern.[4,5] By studying serial samples collected from high-risk individuals undergoing HIV-1 seroconversion, it has been demonstrated that reactivity to p24 (with or without other *gag* or *pol* reactivity) can be the first detectable evidence of exposure to HIV-1 when using Western blot technology.[6] In contrast, an indeterminate pattern without reactivity to p24 has not been associated with seroconversion except in one case in Italy (see below).[7] Therefore, different patterns of reactivity in HIV-1–indeterminate Western blot results may lead to different strategies in notifying and counseling individuals with these test results.

Research studies to assess the significance of EIA-positive, HIV-1–indeterminate Western blot results have attempted to elucidate whether such a result: (1) is indicative of HIV-1 infection; (2) is, in fact, due to reactivity against antigens of HIV-1 and is not a result of a particular laboratory method; (3) is persistent or transient; (4) is indicative of infection with another known retrovirus, such as HIV-2 or human T-cell lymphotropic virus, type I/II (HTLV-I/II); and (5) is related to infection with a currently unknown retrovirus.

FREQUENCY OF EIA-POSITIVE, HIV-1–INDETERMINATE WESTERN BLOT FINDINGS IN BLOOD DONOR POPULATIONS

The frequency of EIA-positive, HIV-1–indeterminate Western blot reactivity in blood donors varies depending on the choice of EIA and Western blot reagents. This is well illustrated by the experience at the American Red Cross, Los Angeles–Orange Counties Blood Services Region, Los Angeles, Calif, as reported in Table 1. In 1986 and 1987, utilizing an EIA test kit prepared by Genetic Systems Corp, Seattle, Wash, and a Western blot with the same HIV-1 antigen, 0.13% of donors had EIA-positive, HIV-1–indeterminate Western blot results. It is of interest to note that these high rates were found when an EIA kit that was sensitive to HIV-1 viral core reactivity was used in conjunction with a Western blot system with an identical antigen. In contrast, using a different set of EIA and Western blot reagents from 1987 through 1989 (EIA from Abbott Laboratories, North Chicago, Ill, and Western blot assay from DuPont/Biotech, Rockville, Md), the annual rate ranged from 0.005% to 0.014%. With the Genetic Systems Corp reagents, 53% of the indeterminate results had a band at p24; subsequently, only 25% of indeterminate results had such a finding. Therefore, the use of differing sets of reagents resulted in a 25-fold decrease in the number of EIA-positive, HIV-1–indeterminate Western blot findings and a more than 50-fold decrease in EIA-positive, HIV-1–indeterminate Western

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Table 1.—Frequency of EIA-Positive, HIV-1-Indeterminate Western Blot Findings in Blood Donors at American Red Cross Blood Services, Los Angeles-Orange Counties Region*

Year	EIA Kit†	Western Blot† Method	HIV-1–Positive EIA Rate, %	HIV-1–Positive EIA Results With HIV-1–Indeterminate Western Blot Results, %	HIV-1–Indeterminate Western Blot Rate, %	EIA-Positive, HIV-1–Indeterminate Western Blot Results With p24 Reactivity, %
1986-1987	GSC	GSC	0.28	48	0.13	53
1987	ABB	DUP	0.09	11	0.01	60
1988	ABB	DUP	0.08	18	0.014	42
1989	ABB	DUP	0.06	8	0.005	25

*EIA indicates enzyme immunoassay; HIV-1, human immunodeficiency virus, type 1.
†GSC indicates Genetic Systems Corp, Seattle, Wash; ABB, Abbott Laboratories, North Chicago, Ill; and DUP, DuPont/Biotech, Rockville, Md.

Table 2.—Studies of Blood Donors With HIV-1-Indeterminate Western Blot Results*

Source, y	Study Site	No. of Donors With Follow-up Sampling
Kleinman et al,[5] 1988	Los Angeles, Calif	150
Jackson et al,[10] 1989	Minneapolis-St Paul, Minn	100
Mozzi et al,[11] 1988	Milan, Italy	64
Lefrere et al,[12] 1988	Paris, France	22
Dock et al,[13] 1988	Syracuse,NY	20
Ferroni et al,[7] 1988	Milan, Italy	16
Van der Poel et al,[14] 1989	The Netherlands	11

*HIV-1 indicates human immunodeficiency virus, type 1.

blot findings with detectable p24 reactivity.

If HIV-1 Western blot testing is done on a blood donor population that is not prescreened by anti-HIV EIA testing, the rate of indeterminate results may be extremely high. For the DuPont/Biotech Western blot assay, licensed by the Food and Drug Administration, the indeterminate rate has been reported as 15%.[8] This high rate far exceeds the rate of HIV-1 infection in the general population, providing evidence that such reactivity is often due to confounding factors.

If HIV-1–indeterminate Western blot reactivity were significantly associated with retrovirus infection, one would expect to see geographic variations in the frequency of such findings. It is difficult to make such comparisons since the HIV-1–indeterminate Western blot rate is so markedly affected by the test systems used in different laboratories. Such a comparison, can, however, be made using information compiled by the National Red Cross Infectious Disease Laboratory Reference Laboratory, which has accumulated data from 56 regional blood service regions.[9] These geographically varied regions use the same manufacturer's EIA kit and ship their EIA-positive samples to the reference laboratory for standardized Western blot testing. It is of note, therefore, that there has been no significant geographic variation noted by region, even when high-incidence and low-incidence HIV-1 regions are compared.

STUDIES OF BLOOD DONORS WITH HIV-1–INDETERMINATE WESTERN BLOT RESULTS

It has been previously established by studies of individuals with HIV-1 risk factors that the Western blot shows reactivity to p24 in the absence of glycoproteins early in the course of HIV-1 seroconversion.[6] To my knowledge, there have been no reports in the literature from studies of persons at high risk for HIV-1 infection that found evolution to a positive Western blot result unless the indeterminate blot showed p24 reactivity.

As indicated in Table 2, at least seven groups of investigators have performed studies that involved follow-up sampling of blood donors with HIV-1–indeterminate Western blot results.[5,7,10-14] Although I will present some of these results in aggregate, it must be recognized that there are methodologic difficulties with this approach since some of the investigators used different HIV-1 EIA and Western blot methods. In total, follow-up samples were obtained from 383 blood donors. Approximately one third of these study subjects resided in areas where the HIV-1 infection rate in the geographic region at the time of sample collection was extremely low. Eight donors developed characteristic positive Western blot results. Seven of these 8 showed p24 reactivity (with or without other bands) as part of their initial indeterminate Western blot results. However, an eighth donor, reported by Italian investigators using their own Western blot antigen preparation, showed reactivity to p17, pr34, and p55.[7] Because these investigators did not use standardized reagents that are routinely used in the United States, I believe this single occurrence is insufficient to alter the conclusion that unless an indeterminate Western blot shows p24 reactivity, it will not evolve into a characteristic HIV-1–seropositive pattern.

Most of these follow-up seropositive samples were obtained from 8 to 32 weeks after the initial indeterminate Western blot results. Because closely spaced serial samples were not obtained from these donors, the results cannot be used to determine the time required to evolve from an indeterminate Western blot result to a characteristic seropositive pattern.

Interviews were conducted with 7 of the 8 seroconverting blood donors. Six were reported to have risk factors for HIV-1 infection. Interviews with 270 blood donors who had persistent indeterminate findings without seroconversion revealed that 8 had risk factors for HIV-1 infection. Therefore, the presence of a risk factor was not a good predictor of whether a given donor with an indeterminate pattern would go on to

Table 3.—Evaluation of Blood Donors With Persistent HIV-1–Indeterminate Western Blot Results*

Source, y	No. of Blood Donors	HIV-1–Indeterminate Western Blot Patterns	Tests Used	Results for HIV-1 Infection
Jackson et al,[10] 1989	91	42 donors with p24 reactivity	Antigen, culture, and polymerase chain reaction assay	Negative
Van der Poel et al,[14] 1989	11	11 donors with p24 reactivity	Culture and polymerase chain reaction assay on cultured cells	Negative
Lefrere et al,[12] 1989	9	7 donors with p24 reactivity	DNA hybridization and polymerase chain reaction assay	Negative
Dock et al,[13] 1988	12	Not specified	Culture	Negative

*HIV-1 indicates human immunodeficiency virus, type 1.

show evidence of HIV-1 seroconversion. The absence of a risk factor had greater predictive value but was still not definitive. These observations are relevant to notification and counseling policies.

ABSENCE OF HIV-1 INFECTION IN DONORS WHO DO NOT DEVELOP CHARACTERISTIC POSITIVE WESTERN BLOT RESULTS

The initial conclusion that HIV-1–indeterminate Western blot findings are not indicative of HIV-1 infection was supported when they did not evolve into characteristic seropositive patterns within 2 to 10 months. However, questions about a prolonged latency period for HIV-1 infection have made these findings somewhat less conclusive than originally thought.[15] Several studies have indicated that individuals at high risk for HIV-1 infection may show persistent Western blot p24 reactivity for months to years at a time when HIV-1 nucleic acid can be detected by polymerase chain reaction assay.[15,16] However, it must be noted that individuals detected in research studies differ from blood donors in that their Western blot reactivity is associated with a negative anti–IV-1 EIA screening result, a situation that would not arise using the blood donor testing algorithm. Nevertheless, confusion caused by such findings has made it imperative to confirm that lack of evolution to HIV-1 seropositivity within an interval of several months reflects the absence of HIV-1 infection. Several investigators have evaluated blood donors with HIV-1–indeterminate Western blot results by direct tests for HIV-1 genomic material.[10,12,14,17] Techniques utilized, as indicated in Table 3, have included HIV-1 antigen tests, HIV cultures, and HIV-1 polymerase chain reaction assay. A total of 111 donors, most of whom had p24 reactivity in the Western blot assay, had negative polymerase chain reaction assay results for HIV-1. These findings, though based on a small sample size, strengthen the conclusion that donors with persistently indeterminate Western blot patterns are not infected with HIV-1.

REACTIVITY DIRECTED AGAINST HIV-1 ANTIGENS IS NOT USUALLY A LABORATORY ARTIFACT

Multiple lines of evidence indicate that a significant percentage of HIV-1–indeterminate Western blot findings are not a result of faulty Western blot assays. Follow-up samples obtained from 250 blood donors showed that 90% continued to have indeterminate Western blot findings at intervals ranging from 2 months to almost 3 years, despite the fact that only 58% of these blood donors had persistently positive EIA test results.[5,10] In one series, 61% of blood donors with HIV-1–indeterminate Western blot results using one manufacturer's antigen preparation demonstrated similar findings when analyzed utilizing a Western blot antigen prepared from a different HIV-1–infected cell line.[5] Test systems that use recombinant HIV-1 proteins as antigens have further demonstrated that, in the majority of cases, indeterminate Western blot reactivity can be attributed to an antibody in the patient's serum that binds to epitopes coded by genes of HIV-1. Three separate investigators have demonstrated that 45% to 89% of indeterminate samples are reactive when recombinant *gag* proteins are used.[13,14,18] It appears, therefore, that such HIV-1–indeterminate Western blot samples can be separated into two categories: (1) Some findings appear to be laboratory artifacts. Reactivity in such samples may be due to the particular Western blot antigen preparation used, the EIA test used, lot-to-lot variation in test kits, or a transient phenomenon in the donor's sera. (2) Some findings are due to reactivity to HIV-1–encoded proteins. Further studies have not established the reason for such reactivity in donor sera.

HIV-1–INDETERMINATE WESTERN BLOT FINDINGS AND THE POTENTIAL FOR OTHER RETROVIRAL INFECTIONS

Because of partial homology and serologic cross-reactivity between the core and polymerase proteins of many retroviruses, it has been hypothesized that blood donors with HIV-1–indeterminate Western blot results may be infected with a different retrovirus.[13] Several investigators have assayed sera with HIV-1–indeterminate Western blot findings for reactivity against HIV-2 or HTLV-I/II.[7,10,12,13,19] While one group of investigators found indeterminate Western blot reactivity for HIV-2 and HTLV-I/II in some donor samples,[13] the remaining groups of investigators found no evidence of HIV-2 or HTLV-I/II infection. No examples of blood donors with clearly positive HIV-2 or HTLV-I/II infection were identified in any of these studies.

It is more difficult to evaluate the hypothesis that HIV-1–indeterminate Western blot findings are caused by cross-reactivity to a so-far unidentified retrovirus. Investigators in Syracuse, NY, have suggested, based on a percentage of HIV-1–indeterminate blood donors giving a history of drinking unpasteurized milk or working on a dairy farm, that such cross-reactivity might be due to exposure to bovine leukemia virus.[13] While such a suggestion is intriguing, it must be pointed out that comparison history data for potential

Table 4.—Notification Strategies for Blood Donors With HIV-1-Positive EIA Results and HIV-1-Indeterminate Western Blot Results*

Group	HIV-1–Indeterminate Western Blot Pattern	Interpretation	Notification and Counseling Strategy
1	p24 plus gp120/160, gp41 plus gp120/160, gp41 only, gp120/160 only	Possibly infected with HIV-1	Notify blood donor of high likelihood of HIV-1 infection, counsel as if donor is infected with HIV-1, obtain follow-up sample for reevaluation
2	p24 only, p24 plus other *gag* proteins, p24 plus *pol* proteins	Possibly infected with HIV-1, but low likelihood	Notify blood donor of low likelihood of HIV-1 infection; the presence of a risk factor for HIV-1 may increase the probability of infection. Obtain follow-up sample for reevaluation. Recombinant protein or synthetic peptide EIA tests on the initial sample may provide additional counseling information
3	p18, p31, p51 p55, p66, and nonviral bands, occurring singly or in combination	Not infected with HIV-1	Notify blood donor that findings indicate an absence of HIV-1 infection, inform donor that he or she is not currently eligible for donation, no counseling or follow-up sampling is necessary

*HIV-1 indicates human immunodeficiency virus, type 1; EIA, enzyme immunoassay.

bovine exposure were not provided for a group of control blood donors with HIV-1–negative findings on EIA.

The best approach to testing the hypothesis of infection with an unknown retrovirus would be to assess whether HIV-1–indeterminate reactivity is transmissible. Two known modes of retroviral transmission are the sexual route and blood transfusion. Therefore, studies of sexual partners of blood donors with HIV-1–indeterminate Western blot results and/or recipients of blood products with HIV-1–indeterminate Western blot results might provide data with which to evaluate this hypothesis. Such studies would require that sexual partner or transfusion recipient samples be evaluated with the same or similar HIV-1 Western blot techniques as were used for detection of the original HIV-1–indeterminate subjects. Mozzi et al,[11] using the DuPont Western blot, evaluated sexual partners of 19 blood donors with HIV-1–indeterminate Western blot results and found 2 with indeterminate reactivity.[11] This 10.5% indeterminate rate is less than the 15% reported in the Dupont Western blot product insert for screening of an anti–HIV-1, EIA-negative blood donor population.[8] These same authors performed HIV-1 Western blot testing on 32 recipients of blood products donated several months previously by 19 blood donors with HIV-1–indeterminate Western blot findings who were HIV-1–negative on EIA at the time of their previous donation. However, they failed to indicate whether any HIV-1–indeterminate patterns were observed in recipients, stating only that there was an absence of seroconversion. A second study of the transmissibility of HIV-1–indeterminate Western blot reactivity to blood transfusion recipients was performed by Van der Poel and colleagues.[14] In this study, 10 recipients of blood products from 6 donors with HIV-1 p24 reactivity on Western blot assay were tested. Seven recipients had negative Western blot results, while 3 had p24 reactivity. However, these 3 recipients had the same p24 reactivity detected in their pretransfusion blood sample. The authors concluded, based on their small number of cases, that HIV-1–indeterminate reactivity does not represent infection with a transmissible agent, such as an unknown retrovirus.

TENTATIVE CONCLUSIONS AND LIMITATIONS OF THE DATA

Observations made in serial sampling of cohorts of high-risk individuals combined with additional observations made in follow-up sampling of blood donors with HIV-1–indeterminate Western blot results have combined to provide a relatively clear picture of the significance of HIV-1–indeterminate Western blot findings. On the basis of these data (not all presented in this review), it seems reasonable to classify HIV-1–indeterminate Western blot findings obtained in asymptomatic populations into three major groups (Table 4):

1. Some band patterns are associated with a high probability of HIV-1 infection. These patterns include p24 plus gp120/160, gp41 plus gp120/160, and isolated gp41 or isolated gp120/160. In fact, according to recent recommendations of the Centers for Disease Control, the first two patterns would be classified as HIV-1 seropositive.[4] While this interpretation is most likely true in the majority of cases, an occasional case has been observed in which a characteristic seropositive pattern cannot be found on follow-up sampling.
2. Some band patterns have sometimes been associated with HIV-1 seroconversion. In asymptomatic populations such as blood donors, while these patterns may occasionally be indicative of seroconversion, they are almost always associated with a lack of progression to a characteristic positive Western blot result.[5] Examples of this type of pattern are p24 alone, p24 plus p55, p24 plus p17, p24 plus polymerase gene products, and other combinations of the same bands.
3. Some band patterns have not been associated with HIV-1 seroconversion in a well-documented serial sampling of seroconverters. Examples of such patterns are p17 alone, p55 alone, p17 and p55, p15 or p55 in association with polymerase gene products, isolated polymerase gene product reactivity, and reactivity at molecular weights not associated with known viral proteins.

Persons who have been identified as EIA-positive and who subsequently show persistent HIV-1–indeterminate Western blot results do not appear to be infected with HIV-1. In addition, there is no evidence to suggest that such individuals are infected with either HIV-2 or HTLV-I/II. The current limited data available from sexual and blood transfusion transmission studies argue against the hypothesis that HIV-1–indeterminate Western blot reactivity is indicative of infection with an unknown retrovirus. While these conclusions are suggested by the available data, it must be recognized that these data do not provide definitive answers. One obvious limitation of the data is the small number of samples that have been analyzed. Second, if persist HIV-1–indeterminate Western blot reactivity is only infrequently associated with infection with HIV-1 or another retrovirus, the association might not have been observed in the reported studies. It is unlikely that this possibility can be absolutely excluded by testing the small number of HIV-1–indeterminate persons in blood donor populations; instead, it would be necessary to test a group of persons at high risk for HIV-1 infection who have persistently HIV-1–indeterminate Western blot results.

DONOR NOTIFICATION

Use of Supplementary Tests to Analyze the HIV-1–Indeterminate Western Blot Sample

Although p24 reactivity is the first sign of HIV-1 seroconversion when the Western blot method is used, it has been demonstrated that antibodies to envelope antigens appear concurrently and can be found utilizing methods that optimize their detection.[20] This observation raises the question of whether the application of an additional confirmatory test to the initial sample could resolve the HIV-1–indeterminate Western blot result. Two such confirmatory tests are radioimmunoprecipitation and immunofluorescence (IF). While it has been suggested that the radioimmunoprecipitation might be definitive in these circumstances, only a few specimens have been tested, and sufficient follow-up data are lacking.[21] Furthermore, radioimmunoprecipitation testing is expensive, and, due to its complexity, not routinely available. Immunofluorescence testing has been more systematically evaluated.[5] In one study, IF was performed on 93 blood donor samples with HIV-1 p24 reactivity on Western blot. The IF test was helpful but not definitive in discriminating the p24 bands associated with subsequent seroconversion from those that remained persistently indeterminate. Results of the IF test were positive in 2 seroconverters and nonspecific in 2 others, whereas findings were negative in 87 nonseroconverters and nonspecific in the remaining 2. Although these results suggest that IF testing may help to determine whether HIV-1 infection occurred in the majority of cases, it must be recognized that this limited experience was obtained using one central laboratory experienced in performing this technique. Adopting IF for more widespread use could be limited by considerations of test performance in less-experienced laboratories and by issues of test standardization.

The most feasible approach for supplementary testing of HIV-1–indeterminate samples is to use EIA methods that employ recombinant envelope protein antigens.[20] This method offers the advantages of ease of performance, a standardized methodology, and potential widespread availability. Nevertheless, relatively few data using this approach have been published. Perhaps studies have been limited by the scarcity of HIV-1–indeterminate samples collected from blood donors later demonstrated to evolve to HIV-1 seropositivity. To date, two groups of investigators have evaluated envelope recombinant assays. They were able to obtain positive results from 4 donors who later went on to seroconvert and negative results from 38 donors who had persistently indeterminate results.[7,18] Many more samples will need to be studied before such an approach can be considered efficacious.

Notification Recommendations

In July 1989, the Centers for Disease Control recommended that persons with HIV-1–indeterminate Western blot results be retested after 6 months, and in the absence of both a characteristic seropositive Western blot pattern and HIV-1 risk factors or symptoms, they "should be reassured that they are certainly not infected with HIV-1."[4]

In my opinion, several important modifications of the recommendations of the Centers for Disease Control should be considered with regard to notification of blood donors. First, the strategy for notifying such individuals should be based on the particular band patterns detected. My approach to notification is outlined in Table 4. Persons with group 1 HIV-1–indeterminate patterns need to be informed of the high likelihood of HIV-1 infection and should be counseled as if they are HIV-1 seropositive. A follow-up sample should be obtained and reevaluated by HIV-1 Western blot.

The approach toward blood donors with group 2 HIV-1–indeterminate patterns should involve a different message than that given to donors with group 1 patterns. These donors should be informed that while there is a possibility that they have been infected with HIV-1, such a conclusion is unlikely in the event they have not engaged in activities associated with a high risk for HIV-1 infection. This message should provide some reassurance to the vast majority of group 2 donors prior to reevaluation of their serologic findings. Follow-up testing for HIV-1 by EIA and Western blot should be performed. Blood centers may elect to assume the responsibility for such follow-up testing or in some cases may urge blood donors to obtain such testing through their own physicians or community resources.

It may be reasonable to further modify the approach to notification of group 2 blood donors based on further evaluation of the initial indeterminate Western blot sample using HIV-1–recombinant reagents. If the donor has negative results for antibodies to both p24 and envelope gene products, the donor could be more definitively reassured that HIV-1 infection is not present. If the donor has positive results for both p24 and envelope gene products, the counseling message should be similar to that for the group 1 donor with indeterminate results. If the donor shows reactivity to *gag* gene products in the absence of envelope reactivity, these findings could be used to provide additional assurance to the donor that HIV-1 infection is not present. However, at present, it would still be necessary to verify this conclusion with Western blot testing on a follow-up sample.

The following approach to notification and counseling of HIV-1–indeterminate group 2 blood donors has been effective at my institution. The donor is notified of the test result at a personal interview session, and the findings are explained with the aid of diagrams and illustrations. A detailed HIV-1 risk factor assessment is performed, and the donor is told that the responses will help to determine the probability of infection with HIV-1. Although the donor is told that no firm conclusions can be drawn until the results of follow-up testing are obtained, the donor is given the most likely interim interpretation of the test results. If there is no admission of HIV-1 risk factors, the donor is told that many donors have had similar findings and that progression to a pattern characteristic of HIV-1 infection has been extremely rare. The donor is advised that, until results from a follow-up sample are obtained, the donor may wish to be cautious in sharing the test results with others, since the potential for misunderstanding their significance is great.

Volunteer blood donors with HIV-1–indeterminate group 3 patterns should be considered to be no different from persons who have anti-HIV, EIA-positive, HIV-1–negative Western blot results. These people should be reassured, based on the test results, that they are not infected with HIV-1. No follow-up testing is necessary. As of September 1989, such individuals are not eligible for future blood donations according to Food and Drug Administration regulations and should be informed of this fact.[22]

A second potential alteration of the recommendations of the Centers for Disease Control would be to adopt a shortened follow-up interval for serologic reevaluation. The choice of a 6-month follow-up interval is most likely based on data that indicate that HIV-1 seroconversion will occur at a mean of 2 to 3 months following exposure and will have almost certainly occurred within 6 months in all HIV-1–infected blood donors. However, these seroconversion data do not pertain to the EIA–positive, HIV-1-indeterminate Western blot serologic findings detected in blood donors but rather to the time for progression from a negative anti–HIV-1 EIA test to a positive HIV-1 Western blot result. It seems reasonable to assume that, if antibody is already present in a concentration high enough to result in a positive anti–HIV-1 EIA test with an indeterminate Western blot result, the interval to development of a positive Western blot would be shorter than 6 months. Published data support this theoretical argument. A panel of sera collected at intervals of several days from four seronegative individuals undergoing HIV-1 seroconversion has been evaluated using EIA and Western blot kits from multiple manufacturers.[23] The maximum period from the earliest positive EIA test to the most delayed positive Western blot result was 4 weeks. These data support the premise that a serologic reevaluation of a blood donor can be performed sooner than 6 months; I recommend 2 to 3 months as a routine policy. Shortening the reevaluation interval is extremely important to lessen the considerable anxiety felt by some persons who are informed that their serologic status with regard to HIV-1 infection is unclear.

A third point of departure from the recommendations of the Centers for Disease Control is whether, after follow-up sampling, donors can be definitively reassured of the absence of HIV-1 infection. The approach adopted is dependent on one's philosophy. My experience has indicated that some individuals have a high quota of anxiety that will not be allayed unless definitive reassurance is provided. I believe that a notification policy should be based on currently known data; since the data indicate that donors with persistent indeterminate findings have not been infected with HIV-1, I believe this message should be strongly stated. If future information shows this not to be the case, notification policies for subsequently identified blood donors with HIV-1–indeterminate findings may be altered to provide a more flexible interpretation of the significance of the findings.

In summary, notification, counseling, and follow-up testing policies for blood donors with EIA-positive, HIV-1–indeterminate Western blot results should be formulated to provide donors with the best possible explanation of the significance of their findings while at the same time minimizing unnecessary anxiety.[24]

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Confidentiality and Informed Consent and the Acquired Immunodeficiency Syndrome Epidemic

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• **The acquired immunodeficiency syndrome epidemic has led to significant medicolegal concerns in the areas of informed consent and confidentiality. These legal concepts are directed at preserving respect for individual autonomy and privacy in the face of the epidemic. Emerging legal doctrines and issues related to consent and confidentiality are outlined, and areas where the laboratory may develop procedures and policies in light of the doctrines are suggested.**

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Among the repercussions of the acquired immunodeficiency syndrome (AIDS) pandemic in the medicolegal arena, questions about informed consent and issues of confidentiality have emerged as significant concerns. Informed consent is an important issue from the time that testing of an individual's human immunodeficiency virus (HIV) status is first proposed. The potential impact of a positive test result on areas ranging from health to employment and insurability makes an informed decision about testing critical. Obtaining appropriate informed consent is also critical when a patient is treated with a therapeutic agent, such as blood or components, that may carry even a slight risk of transmitting HIV.

Like informed consent, confidentiality is an essential aspect of the physician-patient relationship, one made even more important in light of the stigma that may unfortunately accompany a determination of HIV-positive status. Mandatory reporting of the status in many jurisdictions and possibilities that information may be released to insurers, employers, or others have heightened worries about breaches of confidentiality. At the same time, concern about actions that will discourage appropriate use of testing and counseling regarding HIV, including unauthorized disclosure of test results, makes assuring confidentiality an important public health consideration.

A pathologist or other physician may encounter a series of questions with legal implications related to issues of HIV, informed consent and confidentiality, and privacy. One significant set of questions involves the role of the pathologist, the treating physician, or others in securing informed consent before an HIV test is performed. It is critical to determine what informed consent means in this context and to clarify on whom the duty to obtain it rests. Similarly, where treatment is contemplated that carries a risk, however remote, of transmitting HIV, questions arise as to what obligation exists to secure informed consent.

On the other hand, concerns have also been raised about the obligations of the physician to report a patient's HIV-seropositive status. Does the physician ever have a right, or a duty, to inform or warn others about it? Conversely, what duties exist to maintain the confidentiality of that information?

Through an examination of relevant statutes and case law in several jurisdictions, this article offers a preliminary analysis of the legal policies and concerns likely to affect the answers to the questions noted above.

INFORMED CONSENT

The concept of informed consent is rooted in the high value our legal system places on individual autonomy in decision making. As a matter of basic Anglo-

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American law, a physician or anyone else who touches another person without that person's consent, even in the course of administering beneficial treatment, could be subject to liability for the tort of battery. The idea of informed consent goes beyond the protection of bodily integrity and the concept of simple consent; it requires that, in the case of a proposed medical treatment, consent be grounded on disclosure of all reasonably relevant information regarding risks of, benefits from, and alternatives to the proposed procedure. Except in emergencies and a very few other limited situations, the doctrine requires that consent be voluntarily given by a competent patient who has been fully informed of material risks, benefits, and alternatives. Failure to secure appropriate informed consent can in turn subject the physician to the risk of a malpractice claim.

As the court in the landmark case of *Canterbury v Spence* (464 F2d 772 [DC Cir 1972]) noted:

> Every human being . . . has a right to determine what shall be done with his own body. . . . True consent to what happens to one's self is the informed exercise of a choice, and that entails an opportunity to evaluate knowledgeably the options available and the risks attendant upon each.

Part of the underlying rationale of the concept is that the physician has a duty to share his or her superior knowledge; thus, consent may be inadequately informed if the patient has not been given sufficient information. Generally, a treating physician has a duty to communicate any material information about risks of, and alternatives to, a proposed procedure. In determining what is material, courts have adopted one of two approaches, analyzing disclosure either in terms of what information is considered significant in the medical community or, increasingly, in terms of a patient standard, as measured by what a reasonable person in the patient's position would consider significant.

Whatever the standard, failure to have secured adequate informed consent will not ordinarily, by itself, enable a patient to prevail in a malpractice or negligence lawsuit. Under usual circumstances, the patient will also have to show that, had he been given adequate information about the risks, he would not have proceeded with the treatment, and that as a result of the treatment he has been harmed or damaged.

In the context of HIV testing and the laboratory, questions immediately arise as to the nature and scope of the informed consent required for the HIV test. A second important question is who has the responsibility for securing that consent.

With respect to the first question, a few commentators have argued that specific informed consent is not, and should not be, required for HIV testing. The argument advanced is that obtaining consent to draw blood and to perform routine blood tests is sufficient in itself and that there is therefore no need for specific consent for HIV testing. This argument appears to reflect recent practice, as evidenced by one study showing that consent was obtained and documented for only 10% of the tests performed in an urban hospital.[1] The argument is based on the contention that routine medical practice has for years involved a series of blood tests without specific consent for each test and that the HIV test itself ordinarily has no adverse physical consequences for the patient.[2(p36)]

These arguments, however, miss the basic premises underlying the doctrine of informed consent, as well as the obvious social and personal consequences of a positive test:

> If the purpose of the doctrine of informed consent is to place the health care decision with the patient, . . . then serious social consequences are just as relevant for the patient as physical harms. Reasonably prudent patients would regard the potential social consequences of a positive HIV test as highly relevant to the decision to undergo the test. . . . Some patients bear an intolerable psychological burden when informed that they are HIV-positive, particularly if they did not even know that they were being tested.[2(pp37,38)]

Under this rationale, informed consent requires informing the individual of more than the basic facts about the test and the medical implications of a positive result. It likely also requires addressing some of the psychological complications and social and legal consequences of a positive test, including informing the individual whether reporting is mandated and to whom the test result may be accessible:

> The social, emotional and financial risks associated with a positive test result are substantial. Patients may suffer social stigma within the hospital. . . . Since third-party payers generally demand access to the patient's medical records . . . a patient's insurance may be cancelled. . . . his employer may also have access to the information and the patient may lose his job. . . . personal relationships may suffer.[3(p615)]

Presumably for reasons similar to these, legislative or administrative bodies in a number of states, including California, Wisconsin, Hawaii, Massachusetts, New York, Colorado, and others, have enacted laws or regulations requiring informed consent for HIV testing.[3(p612)] Even in the absence of an explicit state statute or regulation, the considerations discussed strongly suggest the advisability of securing specific informed consent before testing.

The appropriateness of this approach is reinforced by the fact that numerous professional organizations that have considered the issue have likewise recommended implementation of a requirement of informed consent for HIV testing. For example, the American Hospital Association, the American Medical Association, and the Presidential Commission on the Human Immunodeficiency Virus Epidemic have all made recommendations that HIV testing be preceded by fully informed consent. The fact that many professional groups have adopted informed consent requirements would be highly persuasive evidence in support of a finding that testing without consent failed to conform to established standards of practice.

Lawsuits seeking damages for failure to secure informed consent, or in fact any specific consent at all, are indeed beginning to be filed. For example, a recent Connecticut case alleges negligence, invasion of privacy, and emotional distress resulting from a hospital's performance of HIV tests contrary to the patient's request that no such tests be performed. Allegedly, the test results were even sent to the patient's employer. In the absence of a statute, the lawsuit seeks relief under common law theories.[4]

Assuming that specific informed consent is required, the numerous risks—

social, legal, and emotional—that may result from the test imply a discussion and counseling process in the course of obtaining informed consent. It is ordinarily the health care professional ordering or administering the procedure who has the obligation to secure the consent. In other words, the duty is that of the treating physician. Whether located in a hospital or a referral laboratory, pathologists are usually physically removed from the site where the blood was drawn; they ordinarily have not established a physician-patient relationship with the tested individuals and usually have no authority over the consent procedure. Under those circumstances, it is doubtful that pathologists have significant legal responsibility with respect to informed consent. However, in some jurisdictions, statutory requirements may be imposed with respect to ensuring documentation of informed consent before testing of a sample is performed. It is unlikely, but not inconceivable, that similar arguments might be advanced even in the absence of statutory requirements. In any case, pathologists may at least consider performing an educational role in terms of assisting other health care professionals in developing and implementing appropriate consent protocols.

In the case of transfusion medicine, applicable professional standards make clear what is in any event also the likely legal norm: informed consent must precede the blood donation.[5] While the physician in charge of the blood bank has a direct responsibility for ensuring that consent procedures are in place in the blood bank, that physician has little or no authority in the other context in which informed consent becomes relevant, ie, prior to transfusion.

In the present climate, the prudent course of action for a physician advising a patient to undergo a procedure that may involve transfusion is to discuss the risks of transfusion-transmitted disease, including AIDS. Claims have already been made in a number of lawsuits regarding transfusion-associated AIDS, based on physicians' alleged failure to warn of the risk of AIDS or advise of alternatives to transfusion.[6] These claims are not likely to succeed if they arise from a transfusion that was performed during the period in which most transfusion-associated HIV transmission occurred, ie, before the risk was known. Nonetheless, with routes of transmission now well established, the wise course is for the physician to disclose the risks and discuss alternatives such as autologous transfusion. Even though the risk may be remote, the seriousness of the disease and the extent of public concern appear to make such disclosure good legal prevention.

As in the case of testing, the duty to secure informed consent lies principally with the treating physician, not with the physician in the laboratory. Here again, however, the laboratory or blood bank physician may be well advised to take an active role in educating other physicians about transfusion risks and alternatives, such as autologous transfusion or directed donation, that might be discussed with patients.[7]

CONFIDENTIALITY

While the concept of informed consent focuses on autonomy in decision making about treatment, confidentiality protections seek to assure that information resulting from those decisions is treated with appropriate privacy. Even if fully informed consent precedes testing, if the test results are confirmed as positive, an immediate concern for both the individual tested and the physician may be the question of access to the test results: To what individuals or entities can or must the results be reported? And, conversely, what protections assure the confidentiality of the information?

An underlying consideration in the area of testing and informed consent is the social consequences that can accompany a positive test result. The stigma and discrimination that have unnecessarily added to the burdens of those infected with HIV likewise make the issue of confidentiality particularly compelling. "AIDS has provoked so much fear, and the groups most at risk are so likely for other reasons to be stigmatized, that physicians must pay particular attention to maintaining confidentiality."[8]

In a majority of states, the fact that an individual has AIDS must be promptly reported to health authorities. Some, but not all, states also require reporting of HIV-positive status. In either case, detailed individual-identifying information may be required by some states, requirements that have been challenged by some commentators on either policy or constitutional grounds. Physicians, health care facilities, and medical laboratories may all be among the mandated reporters. With mandated reporting the norm, and reporting for carrier status as well as for clinical AIDS required in a number of states, the extent of confidentiality protections becomes particularly critical.

Several states have enacted statutes explicitly addressing the confidentiality of HIV information. Most of these statutes provide specific protection for the confidentiality of HIV test results, subject in most instances to limited circumstances in which release of the information is authorized.[9]

The California statutes are one example of this approach. In California, negligent or willful disclosure of test results with individual identifiers, in the absence of express written authorization, subjects the discloser to civil penalties of up to $5000, to liability for damages to the test subject, and to possible misdemeanor liability.[10] There are some limited exceptions to these prohibitions, including a more recent provision permitting physician disclosure to the person believed to be the spouse of an HIV-positive individual. (Blood banks and plasma centers are another exception, with some mandated reporting.) The California statutes also provide for testimonial privileges as to HIV status, for confidentiality of AIDS research records, and for confidentiality of AIDS public health records.

By way of another example, a comparable statute in Rhode Island makes it unlawful for any person to disclose the results of an individual's AIDS test "without the prior written consent of the individual," except for reporting to the health department and certain other authorized releases by health care providers.[11] Among the many other states that have recently passed legislation regulating disclosure of HIV test results are Colorado, Massachusetts, Maine, Hawaii, and Wisconsin. In addition, a number of states have, in response to HIV, amended and strengthened statutes dealing with communicable disease data, sexually transmitted disease data, and/or all medical and health records.

Federal law may also have a bearing on the confidentiality to be accorded to individually identifiable AIDS data, particularly if a federal statute assuring confidentiality is ultimately adopted. The Presidential Commission on the Human Immunodeficiency Virus Epidemic has endorsed federal legislative action to increase confidentiality protections in this area.[12] Federal constitutional and common law protections may also provide a basis for asserting a right to privacy. For example, in one recent case, the court examined, but did not decide the question of, a constitutional right to privacy in information concerning an individual's AIDS status. In at least one other case, a federal court has recognized constitutional rights to privacy in medical records related to HIV.[13,14] Thus, even in the absence of a statute, it is likely that many courts would recognize a basic constitutional or common law right to have AIDS information treated with confidentiality.

Even if a state has enacted new confidentiality provisions, those protections may be subject to exceptions, prominent among which can be an exception for disclosure pursuant to court order. The courts have recently rendered the first decisions specifically addressing disclosure of individual identity in the course of litigation for AIDS-related liability. Most of the decisions to date have occurred in the course of efforts to determine the identity of blood donors. And in that context many, but not all, courts have concluded that confidentiality concerns must be paramount.

In the leading case of *Rasmussen v South Florida Blood Service*,"[15] the plaintiff received transfusions following an automobile accident and subsequently died of AIDS. In a lawsuit against the automobile driver, the estate attempted to prove that the source of AIDS was the necessary medical treatment that was given after the accident. To that end, a subpoena was served for blood service records containing the names and addresses of the donors.

In upholding the blood service's motion to quash the subpoena, the Florida Supreme Court found that the privacy interests of the donors significantly outweighed any utility from the discovery of names in the litigation:

> Disclosure of donor identities in any context involving AIDS could be extremely disruptive and even devastating to the individual donor. . . . AIDS, or a suspicion of AIDS, can lead to discrimination in employment, education, housing, and even medical treatment. . . . By the very nature of this case, disclosure of donor identities is "disclosure in a damaging context."[15]

In addition to holding that the disclosure sought involved constitutionally protected privacy interests, the court also held that the prospect of disclosure would be a significant disincentive to volunteer blood donation. While several other courts, as well as a number of legislatures, have followed similar reasoning in forbidding release of donor identities, other courts have reached a contrary result or have given less extensive protection to the confidentiality interests.

Aside from statutorily required reporting or disclosure in the context of litigation, a physician may face other complex situations in which confidentiality interests have to be balanced against competing concerns. One such issue is the question of access to HIV information about a patient by a health care employee who has experienced an accidental needle stick or other parenteral exposure. A few states have dealt with this issue by statute. In the absence of a statute, two questions arise. The first is whether the patient can be required to be tested, an issue that relates back to the question of informed consent. Arguably, the approach adopted by some hospitals of securing consent to such testing as a condition of admission fails to meet the requirement of voluntary and knowledgeable consent. Even if the patient is willing to be tested, or has already been tested, there may be questions about the scope of permitted information release. Although it can be argued that the information gleaned from a test of the patient is of limited utility to the exposed employee, one approach taken by some states is to allow for the release of data without identification of the patient by name.

Another example of competing concerns is the question of whether the physician should reveal a patient's HIV positive status to the patient's spouse or sexual partner. In the absence of action by the patient and in view of the consequences of HIV transmission, does the physician have a duty to warn or protect this third person that transcends physician-patient confidentiality?[16]

A few states have simplified the legal dilemma by enacting specific statutes. For example, in California it is permissible for a physician to inform the spouse. No civil or criminal liability attaches to physician disclosure of a patient's HIV-positive status to a person believed to be the patient's spouse, so long as disclosure is made in the manner prescribed by statute.[17] (Likewise, the same California statutes permit what is probably implicitly accepted practice in most states: an HIV-positive individual incurs no legal liability by identifying partners to health officials, so that they in turn can inform and counsel the partners.)

In the absence of an express statute, the rationale of *Tarasoff v Regents of the University of California*[18] may provide some guidance. The *Tarasoff* decision imposed a common-law duty on mental health professionals to take reasonable steps to protect identifiable third-party victims of patients believed to pose a serious threat of violence. This duty is viewed as superceding any obligations of physician-patient confidentiality.

It can at least be argued that this rationale should also apply to identifiable contacts of an HIV-positive patient. Some commentators have found a duty to inform on other grounds as well:

> The trend in caselaw has been to place a duty on the doctors of patients with communicable diseases to warn the patients or their families that those in contact with such patients may contract the disease. . . . If the physician knows that the patient intends to engage in sexual relations with a certain person without informing the person that he or she has AIDS and without taking precautions to minimize the risk of transmission, the physician has a duty to warn that person of the possibility of infection from the patient.[19]

Pending explicit statutory or judicial direction, health care professionals must balance the interests involved in particular cases, weighing carefully whether notification is necessary to protect the life or health of specific persons.

While pathologists and other laboratory specialists may not ordinarily be confronted with questions of duties to warn partners, pathologists frequently do have under their supervision and control significant test results on individuals and many employees with access to those results. It is very important to establish clear laboratory policies regarding confidentiality. Even apart from potential legal liabilities, the likelihood of personal embarrassment, stigma, and discrimination resulting from unwarranted disclosure of an individual's HIV status should encourage laboratory directors to assure that their employees appreciate the need for confidentiality and the importance of following laws and procedures to protect it.

CONCLUSIONS

Legal doctrines regarding informed consent and confidentiality seek to protect individual interests in autonomy, dignity, and privacy in the face of the AIDS epidemic. Because the law varies from state to state and is rapidly developing, this article has simply outlined some of the trends. It does not attempt to provide legal advice and is in no way a substitute for consultation with counsel as particular legal questions arise.

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Correction

Incorrect Data.—In the article entitled "A Review of the Histology, Ultrastructure, Immunohistology, and Molecular Biology of Extra-adrenal Paragangliomas" that appeared in the November issue of the ARCHIVES (1989;113:1209-1218), errors appeared in Tables 1 and 2. On page 1212, in Table 1, under the column heading "Chief Cells*," there should be a new line 9, "Leu-7"; line 8 of that column in that table should now read "HISL-19-defined antigen," not "HISL-19-defined antigen Leu-7." On page 1213, column 1, line 14, the correct spelling of the term should be "GFAP," not "G-FAP." On page 1213, in the footnotes to Table 2, the explanatory footnote symbol for the third line of the footnotes should be a double dagger, not a dagger, so that the sentence should read as follows: "‡Study was based on head and neck (branchiomeric) paragangliomas." The next line of the footnotes, "‡Study was based on duodenal gangliocytic paragangliomas," should be deleted in its entirety.

Legal Liability for Transfusion Injury in the Acquired Immunodeficiency Syndrome Era

Thomas F. Zuck, LLB, MD

• The emergence of the acquired immunodeficiency syndrome has wrought changes that have affected not only medicine and science, but many aspects of our social and political structures. In 1983 when it clearly became evident that acquired immunodeficiency syndrome could be transmitted by transfusions of blood components and products, blood banks became the focus of intense scrutiny by the public as well as by the mass media. Suddenly it was known that people could contract acquired immunodeficiency syndrome even though they had not engaged in activities known to place them at increased risk for acquiring infection with the human immunodeficiency virus type 1. In many ways the general fear evoked was, and remains, disproportionate to the risks posed by transfusions. This fear coupled with a general distrust of blood banks may also be reflected in the legal response to people infected with human immunodeficiency syndrome type 1 through transfusions. Further, whether the fault system of our tort law is an appropriate way to determine compensation for people injured by transfusion has been brought into question. For those people injured prior to our recognition of acquired immunodeficiency syndrome, the failings of the current system are most obvious.

(*Arch Pathol Lab Med.* 1990;114:309-315)

Compensation for people injured by transfusions of blood components (This article principally addresses liability of blood centers and transfusion services that provide whole blood and components. The legal liability for commercial fractionaters that manufacture blood products from recovered and source plasma, in contradistinction to whole-blood components, may be different.) generally has been sought on the grounds that a blood center, hospital transfusion service, and/or a physician was negligent in the manner that blood components were provided or transfused—that is, an action in tort. In 1970, an Illinois hospital was held responsible for damages when hepatitis was transmitted by a transfusion.[1] At the time of the transfusion in that case there was no method to determine whether a unit of blood was infected. Nonetheless, the hospital was held responsible for what is termed *strict* or *product* liability. Under this doctrine, sellers of products are held strictly liable for damages caused by use of their products without regard to whether they may be at fault. In reaction to the concerns that providers of blood had no way to protect themselves in this situation, most states passed what are termed *blood shield laws.* These laws recognize that blood is "unavoidably unsafe" and "inherently dangerous" and, therefore, providing blood components is treated as a service rather than as a product sale. Some statutes simply state that providers of blood components can be held responsible for damages resulting from transfusion only if they were due to negligence or willful misconduct.

Negligence actions are based on what the law terms a *cause of action*, which is the theory under which an injured person, or plaintiff, attempts to hold a second party, or defendant, financially responsible for injury. Most jurisdictions require four elements of a cause of action of negligence that must be proven by the plaintiff by a preponderance of the evidence: (1) the defendant owed the

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plaintiff a duty; (2) the defendant breached that duty by either acts of commission or omission; (3) the plaintiff was damaged or injured; and (4) the injury was proximately caused by the breach.

The proximate cause element essentially means that a reasonable person could have foreseen that injury would result if the duty was breached in the manner alleged. As new duties are being defined by the uniqueness of the acquired immunodeficiency syndrome (AIDS) cases, whether a specific outcome could reasonably have been foreseen at the time of the alleged breach, such as secondary infection of a heterosexual partner, may become a major question of fact that must be determined by trial.

Most medical malpractice cases are brought under a negligence theory. The defense usually focuses on proving that there was no breach of duty because a defendant's medical practice met generally accepted standards. In blood banking the regulations and recommendations of the Food and Drug Administration, the Standards of the American Association of Blood Banks, and the directives of the American Red Cross generally reflect the standard of care at a given time. Determining what the accepted standard was may pose questions of fact for a jury and testimony by medical experts on this question may become the centerpiece of the trial.

Some plaintiffs have argued that general negligence standards, in contradistinction to medical malpractice standards, should be applied to AIDS cases. The test of breach of duty in such cases would not be the standard of medical care, but rather what a reasonably prudent layperson would have done in such circumstances. General negligence claims in AIDS cases would probably be difficult to defend because it is unlikely that juries would appreciate the complexities of AIDS and the ignorance about the risks of transfusion early in the epidemic. All transfusion-related AIDS (TRAIDS) cases that have come to trial to date have been brought on grounds of medical malpractice or negligence.

State laws may permit suits against blood banks for "willful, wanton, or reckless misconduct." These causes of action are termed *intentional torts* in that plaintiffs claim that defendants deliberately inflicted injury. Assault, battery, and intentional infliction of emotional distress are examples of intentional torts. These suits may permit assessment of punitive damages, which are monetary awards intended to punish the "wrongdoer." These awards may not be covered by liability insurance because it may be considered against public policy to relieve the wrongdoer of punishment by punitive damages.

Juries frequently appear to feel compassion for plaintiffs and may believe that they can help an infected recipient by finding a blood center liable. Thus, a motion for summary judgment is an important strategy in the defense of TRAIDS lawsuits. In making such a motion, the defendant avers that there are no relevant questions of fact before the court and consequently the court should rule for the defendant without a trial. If granted, this motion blocks the plaintiff from presenting his case to a jury.

It has been stated that TRAIDS cases do not present any legal issues different than those presented by transfusion-transmitted hepatitis, the context in which the law of transfusion-transmitted diseases largely developed. However, the malpractice law as it relates to TRAIDS continues to evolve, especially with regard to the duties owed donors and recipients and what outcomes from human immunodeficiency virus type 1 (HIV-1) transmissions were foreseeable, and by whom, at the time of the alleged breaches of duty. In the balance of this article, the discussion of liability for TRAIDS is divided into periods in an attempt to relate the legal response to the current knowledge and environment of the period. A more complete and detailed discussion of the potential liability of blood centers for transmitting AIDS was published by Lipton.[2]

1977 THROUGH WINTER 1983: THE AGE OF INNOCENCE

The environment was as follows: (1) initial AIDS cases were described; (2) initial cases of AIDS in transfusion recipients were described; (3) infection in hemophiliacs was appreciated; (4) speculations about etiologic agent abounded; and (5) initial response of blood bank leadership was published.

Acquired immunodeficiency syndrome was on us but we were unaware of it until the seminal report from Los Angeles, Calif, and New York, NY, in 1981 of homosexual men with *Pneumocystis* pneumonia and Kaposi's sarcoma.[3] Late 1982, the initial suspected transfusion-related case was reported[4] and data from affected hemophiliacs emerged.[5] In December 1982, the Blood Products Advisory Committee of the Food and Drug Administration (FDA) met at the National Library of Medicine, Bethesda, Md, and discussed the suspicions that AIDS was transmitted by transfusions. Not all attendees were convinced. On January 13, 1983, the American Association of Blood Banks, the American Red Cross, and the Council of Community of Blood Centers issued the first of many joint statements in which they stated that the medical evidence that AIDS was transmitted by transfusion "remains incomplete" but that certain steps should be taken to improve the safety of the blood supply.[6] Because it was known that sexually active homosexual men, intravenous drug abusers, and the sexual partners of these people were at increased risk for AIDS, it was recommended that these people be asked not to donate blood or plasma. But, because the exclusion of certain groups, especially based on sexual preference, could be construed to infringe on their civil rights, and policies were established in ways that attempted to minimize any infringement.

Concurrently in this era, an intense hunt for the etiologic agent of AIDS was underway. Although many postulated it was a virus, it was to be several months before the initial case description of HIV-1, initially termed *lymphadenopathy-associated virus*, was published.[7]

MALPRACTICE CLAIMS

Suits brought by people infected by transfusion during this period generally allege that blood banks should have reacted more quickly and more aggressively. Heavy criticism has appeared in the lay press that leaders in transfusion medicine should have taken more emphatic action (*Saturday Evening Post*. July-August 14-15, 1989). It would be the rare jury member who would not be aware of such criticism. In fact, however, the blood-banking leadership made recommendations 2 months before the Public Health Service,[6] the agency of the federal government that was armed with all the available epidemiologic data about AIDS and TRAIDS.[8]

For cases arising in this era, plaintiffs frequently have attempted to obtain the identity of the implicated blood donor from the drawing facility. Flaws in donor accession procedures and in taking the health history have been sought in efforts to establish negligence. Some courts have held that to encourage blood donations it was necessary to protect donors' rights to privacy and denied requests to identify them by name.[9-11] But, others have found that the rights of the plaintiffs take precedence over those of donors.[12,13] In one case permitting access to the donor, it was noted by the court that the plaintiff believed he may have a cause of action directly against the donor.[13]

The rulings in these cases hinge on the unique facts presented and often are narrow. For example, in *Jane Doe v American Red Cross Blood Services*, SC Region,[14] the court denied the plaintiff access to the donor because the center had been so forthright that the court did not believe any additional valuable evidence would have been made available to the plaintiff by disclosing the donor's identity. Although courts generally have been sympathetic to protecting the confidentiality of donors, it is difficult to predict how a court in a given jurisdiction will rule on a particular set of facts. Blood donor establishments probably would be advised to exert all reasonable efforts to protect the confidentiality of their donors.

It is improbable that physicians, hospitals, or blood centers would be found liable for TRAIDS transmitted by transfusions in this era. Transfusion medicine experts and the Public Health Service were continuing to state that the evidence was "incomplete" and the "possibility" of transmitting AIDS by blood components was "suggested" by sporadic case associations.[8] It would be unreasonable to hold a less expert clinical physician to greater certitude. Thus, even though a transfusion might not have been indicated, it could be argued that the transmission of AIDS could not have been reasonably foreseen as a result of the transfusion. However, it also could be argued that an unindicated transfusion is itself sufficient to give rise to liability for all consequences resulting from the transfusion.

1983 THROUGH 1985 (SPRING): THE GATHERING STORM

The environment was as follows: (1) HIV-1 was identified as an AIDS-causative agent; (2) antibody tests were developed and in clinical trials; (3) self-exclusion by high-risk donors was relied on; (4) a directed-donor joint statement was issued; and (5) selected blood banks initiated: surrogate testing, confidential unit exclusion, look-back on donations by people with AIDS, and patients demanded alternative forms of transfusion-directed and autologous donations and single-donor cryoprecipitate and platelets.

Much of the earlier confusion and mystery of the age of innocence was clarified when HIV-1 (initially called human T-cell lymphotropic virus, type III/lymphadenopathy-associated virus) was identified.[7,15] Thus, it had now become evident that AIDS was a retroviral disease transmitted by exchange of bodily fluids, especially blood and semen. Blood-banking leadership remained unsure what course would be the most prudent to protect the blood supply. The Joint Statement of March 7, 1983 still referred to the "possibility" that AIDS was transmitted by transfusions.[16] Nonetheless, there was broad support for the Public Health Service report that defined persons at increased risk for AIDS and recommended that members of these groups be asked to refrain from donating blood and plasma.[8] Shortly thereafter screening procedures were introduced in donor centers.

Much of the legal controversy in this period revolves on whether surrogate testing would have been effective in improving the safety of the blood supply. Testing for hepatitis B core antibodies (anti-HBc) was considered the most likely candidate, since the mode of transmission of AIDS and hepatitis B were known to be similar. Because the problem of AIDS was so severe in the San Francisco Bay (Calif) area, blood centers located there began testing for anti-HBc in the summer of 1984. It was clearly recognized that even if the testing did not reduce the transmission of AIDS by transfusions, the risks of transmitting hepatitis B could be reduced in this way. Most members of the blood service complex did not believe that anti-HBc testing would be effective and an early pilot study confirmed this belief.[17] Further, it was feared that if plasma from all donors who had anti-HBc were removed from the plasma supply, the concentration and anti-HBs would be negligible and the safety and efficacy of immunoglobulin could be reduced. A study group of the Blood and Blood Products Advisory Committee of the FDA recommended that anti-HBc testing not be initiated[18] and most blood centers followed this recommendation.

One hospital blood bank in northern California analyzed blood donor T-lymphocyte subsets and excluded donors with T-4:T-8 ratios below 0.75.[19] Although this procedure might have been practical in a hospital blood bank, lacking proof of efficacy, it was generally perceived as an unwieldy and impractical way to screen donors in large centers with the technology available in 1984.

While some centers were studying surrogate tests, others were experimenting with confidential unit exclusion.[20,21] This strategy was developed to encourage risky donors who felt pressured to donate to designate their unit as unsuitable for transfusion. It enjoyed only limited acceptance until it was recommended by the FDA in 1986.[22]

When blood centers were notified by health departments that people symptomatic with AIDS or AIDS-related complex had previously donated blood, it was recommended that centers notify recipients of donations from these donors.[23] These programs were initiated as public health measures to prevent further spread of the infection.

In this era, patients became wary about receiving transfusions and demanded alternatives. Blood bankers encouraged autologous donations, particularly prior to scheduled elective surgery. However, patients' physicians showed less enthusiasm about this practice. But it was the directed donor programs that fueled the most controversy. Patients wanted to designate who their donors should be, but early studies indicated that these donors were not safer than donors who gave for the community.[24] Friends and relatives might be pressured sufficiently to give that they would not be able to tell the truth about risky activities. For this reason, a joint statement from the various blood-banking organizations was issued suggesting that directed donation services not be offered.[25] However, it is only recently that, based on hepatitis markers, first-time directed donors (about 80% of directed donors in most programs are first-timers) were found in a large study to be no safer and perhaps somewhat less safe than first-time community donors.[26] Efforts to discourage this practice led to inconsistent policies. Some centers unwisely modified policies to accommodate patients who were sufficiently insistent or prominent. At the request of some patients, cryoprecipitate and platelet concentrates were made from directed apheresis donors in efforts to reduce the number of donor exposures. The extent of these practices nationwide remains unknown, but varied widely.

MALPRACTICE CLAIMS

The malpractice storm was gathering during this period because of indecision about how best to protect the blood supply. Several different strategies were advocated and some were implemented. Despite joint statements, blood bank practice was not uniform. Many knowledgeable and dedicated people differed and it was unclear, and remains so even now, exactly what duty was owed to transfusion recipients by blood centers, transfusion services, hospitals, and physicians. Because one center or hospital performed a surrogate test or used a unique measure to exclude high-risk donors, even though the efficacy of such measures had not been demonstrated, should these practices become the standard of care? If they were, what could be termed a *black sheep* standard of negligence would be created. Not only would blood bank practice become a morass, such a standard of care would be virtually impossible to meet.

In general, plaintiffs have been unsuccessful in claiming that surrogate testing should have been performed, although many of these cases have yet to be tried. In a highly publicized case, *Kozup v Georgetown University*,[27] the Court of Appeals held in granting a motion for summary judgment that the duty owed a recipient would be measured by the professional standard of care that existed at the time of the transfusion. Although the court was satisfied that the standard had been met in that case, it did not specify how the standard had been established. While granting a motion for summary judgment by defendants, in *Shelby v. St. Luke's Episcopal Hospital*,[28] the court rejected the argument that the entire blood-banking industry had adopted inadequate screening measures. Similarly, in *Osborn v Irwin Memorial Blood Bank*,[29] the jury found that the blood center was not negligent in 1983 for not performing anti-HBc testing. A similar result was reached in a Texas case.[30]

One should be cautious about these favorable outcomes. If the consequences are sufficiently dire, negligence could be found even if generally accepted medical practice was followed. In *Helling v Carey*[31] an ophthalmologist was found negligent for not performing a glaucoma test on a patient less than 40 years old even though it was not the standard of care to do so. The consequences of AIDS could be construed to be equally or more dire than blindness, a foreseeable outcome of glaucoma. The court that decided *Helling v Carey* cited a well-known case in which a Federal Circuit Court of Appeals held a tugboat company negligent for not having a radio even though it was not the standard in the industry for tugboats to carry radios at the time the accident occurred.[32] In writing for the Court, Judge L. Hand stated, "Courts must in the end say what is required; there are precautions so imperative that even their universal disregard will not excuse their omission." Most courts have been reluctant to follow the *Helling v Carey* case and it has generally been considered an aberration. In granting a motion for summary judgment, a New York court recently rejected arguments based on *Helling v Carey* and stated that duties owed to blood recipients were based on the professional standards of the industry.[33]

In addition to claims that blood centers and hospitals were negligent in the way they screened and tested the blood, a series of suits have alleged that patients were not adequately informed either about the risks of transfusion or the availability of alternatives. Most of the "duty to warn" cases have been brought against manufacturers of commercial products. Some have been found for the defendant, but settlements preclude accurate assessment of the outcome of many of these cases.

As noted, in the Osborn case Irwin was not found negligent for not doing surrogate testing in 1983, but was found negligent because of its directed donor policies in effect at the same time. At the time, directed donations were discouraged by Irwin, but were offered under certain circumstances that the plaintiff stated were unclear. It is noteworthy that liability was found even though no data were presented by the plaintiff indicating that directed donations would have been safer.

It is difficult to predict the outcome of cases arising in this era because most have not yet come to trial. However, to date, plaintiffs have found many obstacles to finding defendants negligent in the environment of this era. Cases are likely to be decided on narrow grounds and based on unique facts.

1985 THROUGH 1989: THE WINDS OF THE MALPRACTICE WAR

The environment was as follows: (1) HIV-1 antibody testing was initiated and evaluated; (2) donor notification was implemented; (3) look-back programs were started; (4) donor exclusion criteria was refined further; (5) confidential unit exclusion was recommended by the FDA; and (6) a donor reentry algorithm was developed and implemented.

Although the donor self-exclusion measures were generally successful,[34] the safety of the blood supply was enhanced by the introduction of the HIV-1 antibody enzyme-linked immunosorbent assay in early spring of 1985. Nationally, about 4 per 10 000 blood donors were found to have HIV-1 antibodies by supplemental testing, usually Western blot analysis.[35] Two problems emerged early in the testing program that caused blood centers to be reluctant to notify blood donors of possible infection, despite the Public Health Service recommendations to do so.[36] First, the enzyme-linked immunosorbent assay kits were designed to be highly sensitive to provide maximum protection of the blood supply, but they demonstrated poor specificity. With the first test kits, fewer than 5% of blood donors with repeatably reactive screening tests could be confirmed to have antibody by Western blot analysis. Moreover, between 15% and 20% of normal people have indeterminant patterns on Western blot and it was unclear how donors in indeterminant status should be managed. Only recently has the true status of these donors become clarified and it is now known that the great majority of them are not infected.[36-39]

Second, the quality and interpretation of the most commonly used supplemental test, Western blot, varied widely among laboratories and none was licensed by the FDA. These inconsistencies created further confusion as to which donors should be told they were infected. Confidence in Western blot grew as interviews with donors with positive blots suggested the great majority of them had risk factors for AIDS.

Additional concerns were expressed that notification of blood donors with positive test results would prompt people to donate blood to find out whether they had positive test results. Alternative test sites were not available uniformly throughout the country. One news magazine even suggested people donate blood together before having sex (*Newsweek*. November 24, 1986:40) and some states passed complicated laws concerning notification of people with confirmed positive test results.[40] However, by June 1985, sufficient confidence had developed in the testing algorithm, including Western blotting, that a joint statement recommended donor notification and shortly thereafter blood centers began this process.[41]

Recipient look-back posed more difficult problems. When HIV-1 antibody testing was initiated, the transmission efficiency of units that were Western-blot positive or indeterminant was not known, and it was believed that 5% to 19% of people with HIV-1 antibodies would ultimately develop AIDS.[36] Thus, when a blood donor was found to have antibody, notifying the recipient of his prior donations was considered to be a speculative public health strategy. However, concerns about secondary infection continued to mount. In the Tarasoff case,[42] the University of California was held liable because a psychotherapist in its employ failed to warn a murder victim that the therapist's patient intended to commit the murder. This duty was found to take precedence over the confidentiality rights of the patient who became the murderer. A duty to warn a person about the potential for secondary infection with tuberculosis previously had been established.[43] Thus, in the summer of 1986 the blood service organizations recommended look-back to recipients of blood from donors later found to have HIV-1 antibodies.[44] It was also recommended by the Centers for Disease Control, Atlanta, Ga, that all recipients of transfusions between 1977 and the implementation of antibody testing by blood banks in 1985 ask their physicians whether they should be tested for the presence of antibodies.[45] More recently, the Presidential Commission on AIDS affirmed this recommendation.[46]

When a commercial Western blot kit was licensed by the FDA in 1986, donors who had repeatedly reactive enzyme-linked immunosorbent assays but no bands on Western blot were allowed under a strict reentry algorithm to donate again if their enzyme-linked immunosorbent assays were currently negative.[47]

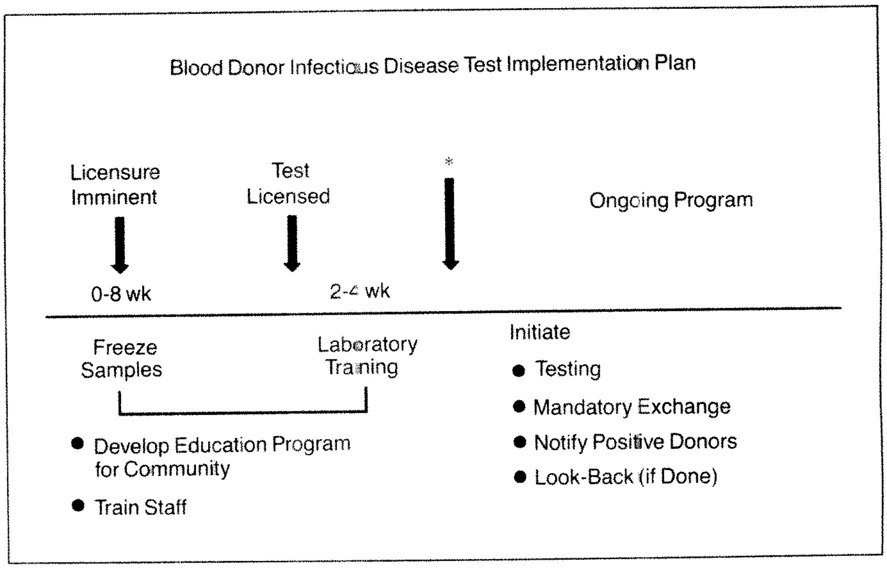
MALPRACTICE CLAIMS

Because of the many complexities surrounding anti–HIV-1 testing during this era, ample pitfalls have been found that could create liability on the part of blood banks if a recipient became infected. The chilling winds of legal battles to be fought already could be felt. One of the more legally contentious aspects of testing has become the manner of its implementation. Two key implementation issues emerged: first, what should be considered a reasonable time after kit availability to train staff and become competent in performing the test? In *KW* v *Belle Bonfilles Memorial Blood Bank*,[48] a jury awarded a patient infected with AIDS $5.5 million (later reduced to an undisclosed amount; both plaintiffs and defendant have appealed) because blood transfused 9 days after the kits were received was not tested. Since it was not possible to test inventory instantly, it becomes an issue for the jury as to how quickly was reasonable. Few centers were proficient in performing the new test and were able to complete inventory testing within 9 days; such a standard is extraordinarily rigorous.

Second, how should inventory be managed in the implementation period during which training and test familiarization is undertaken? In the Carroll case,[49] the jury awarded an infected plaintiff $3.9 million because the Center had not actively exchanged tested cryoprecipitate for untested cryoprecipitate in hospital inventories. (Damages were reduced to an undisclosed amount, neither party has appealed.) Although it had offered to make such an exchange, the Center did not mandate it.

Potential liability also may lie in the manner in which recipient look-back was implemented. Although the HIV-1 antibody tests became available in spring 1985, most blood centers did not initiate look-back for potentially infected recipients until the joint statement was published in June 1986.[44] This could be considered a reasonable starting date. However, because at least one center started recipient look-back in late summer 1985 (F. Morales, MD, personal communication, 1989), it could be alleged that this earlier date should be the standard and hospitals should be held responsible for not having initiated look-back prior to the joint statement. These cases could be particularly difficult should secondary infection of sexual partner(s) of an infected recipient have occurred.

Additional causes of action may be built around failure to implement more intensive donor interviews to improve the rate of self-exclusion and confidential unit exclusion. This issue has come to the fore as studies have documented delayed seroconversion in gay men,[50,51] and exit questionnaires of blood donors indicated incomplete understanding of the AIDS material (T.Z., Terry M., unpublished data, 1989). Two approaches have been advocated. First, donors are questioned directly about whether they engaged in risky behavior. Recent stud-



Key tasks in a plan to implement a new blood donor screening test for an infectious disease. The times are arbitrary and should be dictated by the logistics of the test, the type of center operation, and other factors that may be unique to either the center or the particular test; the rationale should be documented. Asterisk indicates day 1 of ongoing program.

ies have suggested donors accept such questioning.[52] However, the efficacy of this approach has not been demonstrated and there are concerns that donors may not be forthright in the donor room environment.

Second, donors are asked whether they understand the AIDS material they are asked to read, that they may be infected and still have a negative test result, and how to use confidential unit exclusion. This approach is designed to guide and encourage people at risk to exclude their units; however, the validity of this approach has not been proven.

As indicated, the potential for liability in this period is significant, but few cases have come to trial. Both the Carroll and KW cases, especially the latter, suggest that juries are sympathetic to the plight of infected recipients and their sexual partners. Appellate courts may be sympathetic to plaintiffs as well. It was recently held in New York[53] that blood components were included under a toxic substance act that permitted damaged parties to sue even though the statute of limitations had expired. In most states such statutes limit the interval between the alleged negligent act and the filing of the suit. The court permitted plaintiffs to avoid the limitation by applying a law designed to deal with inapparent environmental hazards. Thus, the future of suits arising from actions during this era is clouded at this time.

1989 AND INTO THE FUTURE: THE STORMS REGATHER

It is, of course, not possible to predict what events will drive future liability in the AIDS area. However, as plaintiffs have had difficulty proving negligence in most cases, more novel causes of action are appearing. Several of these claim intentional torts. In the Kozup case (see above) the appellate court sent the case back to the trial court to try an assault claim. Although the case was decided for the defendant (F. O'Brian, JD, personal communication, 1989), a question of fact was raised about assault.

In a more disturbing case, *Roe v United Blood Services Inc* (S. Labensky, JD, Council of Community of Blood Centers interim meeting, Milwaukee, Wis, 1989), it was claimed that a delay of over 1 year in look-back to a potentially infected recipient supported a claim of "intentional infliction of emotional distress." One of the elements of this intentional tort is that the defendant's behavior was an "outrage." This case was settled recently for an undisclosed amount.

The American Association of Blood Banks, American Red Cross, and Council of Community of Blood Centers have been sued in a Texas court for engaging in a "conspiracy" to withhold the "true" risk of blood transfusions from the public and to "block" the use of surrogate tests. This case will be moved to federal courts because the American Red Cross will not accept service in a state court. The potential outcome of this case is unclear, although settlement is not possible with this allegation.

It appears wise for blood centers and transfusion services to learn from the AIDS cases decided to date. As new tests emerge, a precise implementation plan should be developed with consultation from risk managers (Figure). This plan should include establishing a frozen repository of donor samples, an education program for community physicians and center staff, technical staff training schedules, and implementation dates for the various steps in the plan. The rationale for the timetable should be documented. When it is known that a new test is to be implemented soon, a frozen repository should be established as early as possible to permit the most rapid possible testing of inventory. It has been suggested that blood centers notify physicians that implementing a test is imminent so elective surgery may be delayed or patients given that option. As the testing program begins, unit quarantine, physical exchange of inventory, donor notification, and look-back, if it is to be initiated, should all be implemented simultaneously.

In addition to planning specific test implementation, excellent general preventive strategies for blood centers and transfusion services have recently been outlined by Fleisher.[54]

Finally, current malpractice law appears poorly suited to accommodate patients infected with HIV-1 by transfusions, especially early in the epidemic when no party seems to be clearly at fault. Both juries and courts may become understandably sympathetic to the TRAIDS victims. The risks of transfusions are not unlike the risks of vaccines—they cannot be reduced to zero.[55] But, as the manufacturers of vaccines have been protected from ruinous liability suits with a federal compensation pool, so could blood centers. A way to compensate all infected recipients, even if no fault should be found. It could be argued that it is poor public policy to allow the current adversarial system based on fault to determine compensation in TRAIDS cases. Alternative dispute resolution methods should be developed and perhaps compensation pools established. It is probable that additional infectious agents transmitted by blood transfusions will be discovered. It would appear appropriate to develop a generic solution to compensation of recipients who become infected by transfusions. Perhaps the development of such systems could serve as a legacy left by the crisis of TRAIDS.

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Physician Education

The Challenge of Acquired Immunodeficiency Syndrome

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• **Acquired immunodeficiency syndrome is a major health problem confronting society. Our communities are looking to physicians to help in both educational efforts, as well as in formulating public policy in the fight against the disease. For physicians to fill this important role, they first must be knowledgeable themselves. There is strong evidence that many physicians have resisted learning about acquired imunodeficiency syndrome. The increasing number of infected individuals requires the medical profession to find ways to overcome the barriers that prevent our colleagues from acquiring needed information. We must understand the reasons for our educational failures and devise methods to reach physicians with the knowledge base that they will need in helping to combat this terrible disease. Pathologists are in a particularly good position to help in this educational process.**

(*Arch Pathol Lab Med.* 1990;114: 316-318)

If you think education is expensive, try ignorance.

Derek Bok

Acquired immunodeficiency syndrome (AIDS) is a new and deadly disease that has already killed tens of thousands of people and has infected millions of others. A diagnosis is possible, but treatment is in its infancy and a cure does not exist. This is a serious health problem, and it is being made even worse by the poor response of a large segment of the medical community. The disease has, within a decade, produced fear and panic among the health care professions, as well as the public. It will continue for the foreseeable future, and no physician will be able to avoid dealing with the disease.

A common theme from the beginning of the epidemic has been that education is the only effective weapon that we have in preventing the continued spread of the disease. The public has and will continue to look at physicians as the primary resources of accurate information on AIDS. For physicians to fill this important task adequately, they must be educated themselves.

DO PHYSICIANS WANT AND NEED AIDS EDUCATION?

Numerous studies have shown that most physicians desire more information on AIDS, and this is fortunate, because other studies have demonstrated many physicians are both ignorant of many basic facts related to AIDS and inhibited through fear and/or a lack of compassion from even discussing the topic with patients.[1-14] Charles Mayo once commented that there are two purposes to medical education: (1) to help those who are ill and (2) to advance the science.[15] Acquired immunodeficiency syndrome education for physicians certainly fulfills these two goals. However, it is clear that some physicians have decided that AIDS is different and have ignored the disease. Some have decided that patients infected with the human immunodeficiency virus (HIV) are not worthy of their care or concern. Many physicians seem to lack even a simple scientific curiosity for a novel new disease. These factors pose problems for educators, for to be successful, adult education must have students who are willing participants and have an interest in the subject at hand.[16,17]

WHY IS THERE RESISTANCE TO HIV INFORMATION?

Physicians are trained to be skeptical of new scientific findings, and through history, the medical profession has never been quick to accept new ideas even when they seem obviously good to the proponents. One need only remember Hippocrates, Galen, Harvey, Semmelweis, Lister, and scores of other medical educators to see that the job of educat-

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ing physicians about new and controversial topics has never been easy.[18]

Acquired immunodeficiency syndrome is no different. Common problems in getting physicians to participate in any educational program are time constraints, the topic as it relates to each physician's specialty, a physician's perception of his or her need for the information being presented, easy access to the information, concise presentation, and whether official continuing medical education credits are given.[16] Acquired immunodeficiency syndrome also has special problems, among which are physicians believing that they will not have to "deal" with the problem, their fear that they will be seen as "AIDS doctors" which may endanger their practice, their mistrust of much information because they see the source of the information (the government) as politically motivated, their discomfort with the intimate sexual aspect of the disease, and the hostility of many physicians toward those persons currently most affected, ie, the homosexual and intravenous drug user. These problems can and must be overcome if the medical profession is to take a leading and responsible role in the battle against the disease, but the challenges are great.[1-14]

AIDS: WHAT SHOULD PHYSICIANS KNOW?

Physicians are a diverse group of individuals who practice a variety of specialties in a variety of settings. Nevertheless, patients tend to see all physicians as the same, at least with regard to their general medical knowledge. Thus, every physician regardless of specialty, will be asked questions pertaining to AIDS, and they must be able to answer these questions professionally and factually.

What should every physician know about AIDS? There have been a number of published recommendations on what basic information should be included in AIDS education for physicians. This should include a brief update on the virus, the clinical spectrum and course of the infection, epidemiology, how to recognize high-risk behaviors and at-risk patients, how to provide AIDS preventive messages, how to counsel and utilize HIV serologic testing, how to identify community-based resources for HIV-infected patients, and how to deal with physician discomfort and/or negative attitudes toward those persons infected or at risk.[3-14] Each of these topics could be expanded at length, and there are scores of other subjects, for example, the use of lymphocyte subset testing, the microbiology of AIDS, treatment protocols, blood bank issues, and universal precautions in the hospital and office setting.

HOW SHOULD THE INFORMATION BE COMMUNICATED?

Educational efforts must be professional and unbiased, and one must be prepared to deal effectively with hostile questions in a nonjudgmental fashion. One of the most important goals of all medical education is to encourage listeners to pursue the subject further on their own as a result of their own intrinsic interest in the topic under discussion. The mission of the educator then is to renew the scientific curiosity of the physician to learn more about AIDS despite any personal inhibitions that he or she may have as a result of how the disease is spread or who the primary persons infected are at this time. Compassion for those persons who are ill remains one of the honorable attributes of medicine in the caring for all those who are sick.

THE PATHOLOGIST ROLE IN AIDS EDUCATION

The pathologist is in an ideal position to become an active AIDS educator. Pathologists in most settings are already looked to by their hospitals and medical colleagues as resources in providing a wide range of educational programs on medical topics. Pathologists are also already intimately involved with many aspects of HIV infection, including serologic testing, lymphocyte subset typing, blood bank issues, and diagnosis of infections through microbiology, cytology, surgical pathology, and the autopsy. There are many ways for pathologists to convey HIV information to physicians. Although the lecture format is the traditional way, other modes can be effective and are particularly useful for the pathologist: laboratory newsletters, comments placed on relevant laboratory and/or pathology reports, clinical conferences, and locally produced videotapes. Physicians obtain a large percentage of their continuing medical education from informal sources, namely, their own personal patients, ie, their "experience" and consulting with their colleagues. Therefore, the nonlecture modes of transmitting knowledge (ie, the curbside consult) may be most useful and practical.

HOW CAN THE EDUCATOR KEEP UP TO DATE?

For physicians to educate other physicians, they must first become knowledgeable themselves. Volumes of material are now available on AIDS. But frequently the newest and most controversial data are presented and widely distributed via the media before its publication in peer-reviewed journals. Surveys have shown that physicians, like the lay public, obtain most of their AIDS information via the media.[5] The educator must therefore be alert as to what is in the media and be prepared to balance that information with what is documented in the medical literature. This is not always easy, but many excellent resources are readily available, including the Centers for Disease Control weekly *Morbidity and Mortality Weekly Report*, and numerous journals, such as the *Journal of the American Medical Association*, *New England Journal of Medicine*, and *Annals of Internal Medicine*. Several computer databases, such as MEDLINE, carry extensive information on AIDS that is accessible for a small fee. A few of these should be chosen, and the radio and television should be monitored to be responsive to public concerns.

THE MOST COMMON EXCUSE FOR IGNORING AIDS EDUCATION

Information on AIDS does change, and a difference of opinion among experts is common as it is in many areas of medicine. Regardless, physicians frequently become frustrated, and it is not unusual to hear physicians say that they will wait to learn about AIDS when "more is known and the experts can agree." They must not wait, because critically important information on HIV infection is already well known and has been widely accepted for several years, and valuable time is being lost. The causal agent, modes of transmission, high-risk groups and behaviors, serologic tests, clinical presentation and course, and risks of transmission to health care workers have all been extensively covered in the medical literature, and broad agreement exists. Treatment protocols and new diagnostic tools will continue to evolve.

DEALING WITH CONTROVERSIAL ISSUES

Issues, such as mandatory testing, informed consent for testing, anti-discrimination laws, and myriads of other legal, social, economic, and political aspects of AIDS are important and generate large amounts of heat. They are interesting to debate and should be acknowledged as important, but if not handled carefully, they can destroy the ability to convey medical and scientific facts to the audience. One must separate the issues into medical and social aspects of the disease and not let discussion of the "hot" social aspects inhibit the listener from hearing the medical information. If the physician has views on the social aspects that are not acceptable to the audience, his or her credibility on the other aspects of the disease will be lessened. Notwithstanding, the presentation must convey compassion.

CONCLUSION

The need for aggressive and continued AIDS education of physicians has never been greater as the infection has not yet peaked, and all physicians will need to have a sound knowledge base of HIV infection. Pathologists are in an ideal position to help lead the fight against the ignorance of this terrible disease.

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In Other AMA Journals

JAMA

Non-A, Non-B Hepatitis and Antibody to Hepatitis C Virus
J. W. Mosley; R. D. Aach; F. B. Hollinger; C. E. Stevens; L. H. Barbosa; G. J. Nemo; P. V. Holland; W. H. Bancroft; H. J. Zimmerman; G. Kuo; Q. L. Choo; M. Houghton (*JAMA.* 1990;263:77)

Countdown to Millennium—Balancing the Professionalism and Business of Medicine
G. D. Lundberg (*JAMA.* 1990;263:86)

The Approach of a New Millennium
L. S. King (*JAMA.* 1990;263:88)

The Epidemiology of Fear

Scientific, Social, and Political Responses to the Occupational Risk of Blood-Borne Infection

Paul Bachner, MD

• Pathologists will have an important role in implementing safety precautions to prevent transmission of blood-borne diseases in the laboratory and elsewhere in the healthcare setting. This article reviews proposed professional and regulatory solutions in the context of widespread public and professional fear concerning occupational transmission of human immunodeficiency virus and hepatitis B virus. The proposed rule of the Occupational Safety and Health Administration of the Department of Labor, Washington, DC, is described in detail.

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The facts concerning documented and potential transmission of the human immunodeficiency virus (HIV) have been succinctly stated by the American Academy of Pediatrics[1]: (1) HIV has been documented to be transmitted in three ways, sexual intercourse, parenteral inoculation of blood, and congenital or perinatal transmission from a woman to her fetus or newborn; (2) no studies have reported transmission by urine, feces, saliva, tears, or sweat; (3) the risk of infection to healthcare workers who are taking care of patients with acquired immunodeficiency syndrome (AIDS) or persons infected with HIV is extremely low; and (4) studies of household contacts of AIDS have failed to document significant infection transmission. Despite these facts, the public at large and many physicians and healthcare workers appear to believe, as judged by their actions and statements, in the possibility of casual, environmental, and nosocomial transmission of HIV in the absence of documented vectors of infection and transmission.

Although data demonstrating a low risk of nosocomial transmission in the healthcare environment have been widely disseminated,[2-5] a high level of concern persists and has generated some highly unusual suggestions, including the quarantine of patients and the refusal to treat them. These concerns arise against a background of persisting, widespread, and deep professional and societal fear about the mode of spread and the contagiousness of AIDS and HIV infection.

Factors contributing to these fears include, in addition to the large numbers of HIV-infected individuals and patients with clinical AIDS, inadequate knowledge about modes of transmission, evidence demonstrating worldwide progression of HIV positivity into heterosexual populations, the apparent high likelihood or inevitability of disease and inexorable progression to death subsequent to infection, the lack of a vaccine and effective therapies, as well as the association of AIDS with stigmatized groups and abhorrent behaviors.

Several studies have attempted to identify specific psychodynamic and psychosocial causes of what appears to be an exaggerated fear of occupational transmission of AIDS.[6-8] The threat of AIDS has been shown to produce an affective stress response characterized by avoidance, extreme precautions, and verbal expressions of fear regarding the disease.[9] A small minority of healthcare workers have refused to treat patients with AIDS, and some institutions that are known to care for patients with AIDS are being avoided as training sites for the health professions as well as by patients.[10] These concerns have been particularly voiced by certain groups, orthopedists and cardiac surgeons, who face repeated and massive exposures. They believe that the "best guess" estimates of 0.5% risk of infection after parenteral exposure represent a real, if not large, risk.

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An interesting perspective on these issues is provided by the new discipline of risk assessment, which attempts to aid in the process of identifying, characterizing, and quantifying risk.[11] Psychophysical scaling and multivariate techniques are used to produce quantitative representations of risk attitudes and perceptions. Thus, injury from alcohol or snowmobiles, for example, constitute "acceptable" hazards for most because they are controllable, neither dread nor global, equitable, and voluntary. Hazards that are clearly highly unacceptable such as nuclear reactor accidents and toxic chemical pollution are classified as dread, global, inequitable, catastrophic, involuntary, and uncontrollable. It can be seen intuitively that the risk of nosocomial and occupational transmission of AIDS would readily fall into the latter category.

The response to the concerns and fears about potential occupational or nosocomial transmission of HIV and hepatitis B virus has been the development of many, and at times disparate, recommendations, guidelines, and practices promulgating infection controls directed against occupational transmission of blood-borne disease. These have originated from individuals, professional organizations, and local and federal government.[12-15]

The May 1987 Centers for Disease Control, Atlanta, Ga, recommendations[14] have achieved de facto regulatory status because the Occupational Safety and Health Administration (OSHA) mandated (October 19, 1987) compliance with the Centers for Disease Control guidelines under the temporary enforcement authority available to it under the general duty clause of the Occupational Health and Safety Act of 1970. Subsequent inspections performed by OSHA categorized two thirds of the 60 000 healthcare workers monitored as being in compliance with the guidelines; 400 violations were identified and penalties levied against facilities predominantly for failure to adhere to personal protection requirements, housekeeping requirements, waste disposal, and failure to provide training and education.[16]

On May 30, 1989, the long-anticipated *Proposed Rule and Notice of Hearing for Occupational Exposure to Blood-borne Pathogens* was published in the *Federal Register* by OSHA of the Department of Labor.[17]

The new rules are based on the Centers for Disease Control guidelines but add a layer of complexity and specificity of practice that will have a profound and dramatic impact when the new rules become final. They will have the weight of law and, by virtue of stringent requirements impacting all phases of laboratory practice, will have major consequences for all healthcare facilities, clinical laboratories, and their employees. The broad mandate of the rules have been extended beyond the traditional borders of "healthcare" and are to be "based on occupational exposure to blood and other potentially infectious materials, whether or not the individual is employed in the healthcare industry."

OSHA PROPOSED RULE FOR OCCUPATIONAL EXPOSURE TO BLOOD-BORNE PATHOGENS

General provisions of the rules include the following requirements for employers to (1) provide a workplace free of hazards, (2) provide education and training, (3) evaluate all procedures in regard to potential infectious agent exposures, (4) identify all workers with potential hazardous exposures, (5) implement signage and labeling, (6) implement universal precautions, (7) make available personal protective equipment such as gloves and barrier protections, and (8) make hepatitis B vaccine available to all occupationally exposed employees.

HISTORY OF OSHA PROPOSED RULE

The history of the development of the rule reflects strong political pressure exerted by organized labor that included an initial request for an emergency temporary rule to protect workers from HIV and hepatitis B. Emergency rule making was rejected by OSHA, but the proposed standard was issued in January 1989. Some public commentary has been received and the rules have undergone clearance at both the Department of Labor and the Office of Management and Budget.

It is anticipated that following the commentary period and public hearings scheduled for Washington, DC, Chicago, Ill, and San Francisco, Calif, in the fall of 1989, a final rule will be adopted within 1.5 years. In the interim, OSHA will continue enforcement and inspection based on existing enforcement guidelines. Since 1987, OSHA policy has held employers responsible for failure to implement the Centers for Disease Control recommended guidelines. The OSHA's authority stems from Section 5(a)(1), the "General Duty Clause" of the Occupational Safety and Health Act of 1970, which requires that "every working man and woman must be provided with a safe and healthful workplace."[18] The document published in the *Federal Register* consists of 97 pages of extensive commentary and supporting documentation for the six-page proposed rule. The College of American Pathologists, Skokie, Ill, has responded in detail to the rules; although long and difficult to read, every pathologist who is concerned with the impact of these regulations on laboratory practice should be familiar with them.

SUMMARY OF PROPOSED RULE

The proposed rules will require the following of the employer: a written infection control plan that identifies and documents employees and positions with occupational exposure *without regard to the use of personal protective equipment.* If "reasonably anticipated duties" may result in occupational exposure, a written infection control plan must be designed to minimize or eliminate exposure. The plan shall contain, at a minimum, schedules and methods of implementation, and shall be reviewed and updated as necessary.

Methods of compliance that are mandated include universal precautions, engineering and work practice controls, the provision and use of personal protective equipment, and specific and rigorous housekeeping procedures.

One of the most significant provisions of the rule is to require the employer to make available hepatitis B vaccination to *all employees who have occupational exposure on average one or more times per month.* The employer is also required to provide post-exposure evaluation and follow-up in the event of an exposure incident.

Strict demands for record keeping,

requirements for signs and labels, and information and training programs "appropriate in content and vocabulary to educational level, literacy, and language backgrounds of employees" complete the mandate of the proposed rules.

COMMENTARY ON PROPOSED RULE

The specific intent of the proposed rules is to reduce the risk of transmission of HIV and hepatitis B among the nation's estimated 4.7 million healthcare workers and those who are employed in law enforcement, fire and rescue operations, blood banks and research laboratories, correctional facilities, and the funeral industry. The rules are reflective of high levels of concern about AIDS transmission in the healthcare environment, despite the fact that only approximately 25 documented cases of HIV seropositivity have been documented in healthcare workers subsequent to apparent occupational transmission. This anxiety persists despite the absence of evidence of environmental transmission of the HIV virus and summarized data from a number of prospective studies that demonstrate a low relative risk for nosocomial and occupational transmission of HIV.[5]

In a total of 10 longitudinal studies, 6 seroconversions were recorded from a total of 1408 percutaneous exposures. Seroconversion was not recorded in the absence of parenteral exposure or other conventional risk factors. The failure to demonstrate seroconversion in household contacts of HIV-infected individuals except in children of infected mothers and in sexual contacts has been cited as evidence against casual nosocomial or occupational transmission.

COMPLIANCE REQUIREMENTS

The OSHA defines universal precautions as "a method of infection control in which all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV and other bloodborne pathogens." It requires that "universal precautions shall be observed to prevent contact with blood and other potentially infectious materials, unless those precautions would interfere with the proper provision of healthcare or public safety services in a particular circumstance, or would create a significant risk to the personal safety of the worker." The exemption for delivery of healthcare or public safety reasons has elicited vigorous protests from representatives of organized labor.

Universal precautions have been generally adopted by the healthcare community,[19] but discussion and controversy about the wisdom and applicability of this policy continue.[20,21] The public health community and many physicians with extensive infection control and AIDS experience believe that universal precautions represent the best strategy to minimize occupational risk and to protect against all nosocomial pathogens[22]; nevertheless, some contend that the inflexible application of universal precautions is costly, may place healthcare workers at an added and unnecessary risk, and is unpalatable to healthcare workers in the face of known infection, particularly in low prevalence settings.

Mandated methods of compliance (universal precautions, engineering and work-practice controls, and personal protective equipment requirements) are likely to be the most controversial and expensive of the OSHA regulations.

The rule specifies circumstances in which gloves, eye protection and face shields, and protective body clothing must be provided by the employer and used by laboratory and phlebotomy personnel. General agreement exists that gloves are required for anticipated contacts with blood and body fluids and it has been shown that, at least for dentists, the routine use of gloves is associated with a decreased risk of acquisition of hepatitis B seropositivity.[23]

The need for careful attention to needles and sharps as well as frequent handwashing is well established. The former represents the most obvious and quantitatively the most important source of potential occupational transmission of HIV and hepatitis B virus. Of 25 cases of HIV infection associated with occupational exposure cited by OSHA in the *Federal Register*, 18 (72%) occurred by needlesticks (16) or cuts with sharps (2). The remaining 7 exposures are assumed to have occurred via mucous membrane or non–intact skin exposure.

However, an area of concern to pathologists will be the largely unproven value of barrier protection methods in hospitals and laboratories.[24] A review of the proposed rules will demonstrate an excessive and rigid emphasis on the mandated provision and use of barrier protections and personal protective clothing (eg, fluid-proof aprons, gowns, gloves, masks, face shields, head and foot coverings) when "there is a potential for occupational exposure." The use of potential in this context is far too broad and inclusive unless a qualifier such as "unavoidable potential" or "likelihood" is added, thus allowing for the exercise of some degree of flexibility and judgment in implementation based on specific and local circumstances.

HEPATITIS B VACCINATION

Paradoxically, although a safe and effective vaccine for hepatitis B has been available for many years, it is estimated that 18000 healthcare workers are infected with hepatitis B each year with an estimated 200 to 300 annual deaths from complications. It is further estimated that less than 30% of healthcare workers at risk avail themselves of this vaccine. The suggested reasons for the poor utilization of vaccination include cost, concerns about the safety of the vaccine, limited knowledge about the risks and consequences of hepatitis, poor motivation, and the absence of vaccination programs.[25,26]

I believe that most pathologists will be in favor of the OSHA requirement for the employer to make hepatitis B vaccination available but many will question the apparent mandated provision of prevaccination testing of antibody status as expensive and not necessary.

BIOHAZARD LABELS

Proposed requirements for signs and labels promise to be a source of confusion, controversy, and expense. The rules require that "warning labels shall be affixed to containers of infectious waste; refrigerators and freezers containing blood and other potentially infectious materials; and other containers used to store or transport blood or other potentially infectious materials...." A literal interpretation would require all equipment (refrigerators, transport carts, test tube racks) to have the universal biohazard symbol, adding no measure of safety and diluting the impact of the symbol (Hoeltge G.A., personal communication, 1987).

COST CONSIDERATIONS

The OSHA has provided an estimate of anticipated costs including an average annual cost per facility of $1373, with an estimated average cost per hospital of $32 875. Sources outside of OSHA believe that the published estimates are far too low, with documented increases in hospital costs of 15% and increases of up to 50% in glove costs alone.[22] Although studies in non-AIDS hospitals have looked at aggregate costs for the care of patients with AIDS,[27] only a limited number of studies have attempted to look at the incremental costs of implementation of universal precautions and infection control practices directed at minimizing the potential for occupational transmission of HIV and hepatitis B virus.

At the request of the AIDS Task Force of the College of American Pathologists, R.D. Smith, MD (personal communication, 1987) reported the results of a study that was performed at the University of Cincinnati (Ohio) Medical Center. A calculated incremental phlebotomy cost of 83 cents per phlebotomy was demonstrated based on an average annual cost of $100 000 ($700, bed inpatient service; $500 000, outpatient and emergency department visits; and $120 000, phlebotomies).

Whatever the cost, it should be remembered that laboratories in general and hospital laboratories in particular will be unable to recover most of the incremental costs because of fixed and declining laboratory and hospital reimbursement rates.

AUTOPSY AND SURGICAL PATHOLOGY

An area of particular interest to pathologists is that of safety considerations in anatomic pathology in general and in the performance of autopsies in particular. The College of American Pathologists has prepared a video tape on the subject of "safety precautions for the high-risk autopsy"[28] and the Tentative Guideline (M29-T), *Protection of Laboratory Workers From Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*[15] contains detailed recommendations on the processing of clinical laboratory, autopsy, and surgical specimens.

Some pathologists have expressed a reluctance to perform autopsies on known AIDS patients or will only perform limited autopsies in these cases. The reasons cited have included an unwillingness to expose themselves and those assisting at the autopsy to a perceived excessive risk as well as the inability to bring appropriate resources to bear in the smaller community-based facility. Other pathologists emphasize that HIV acquisition has never been demonstrated as a result of autopsy exposure and believe equally strongly that pathologists have an obligation to perform autopsies on patients with AIDS, and that such autopsies, and indeed all autopsies, can be performed safely with the exercise of prudent and reasonable care.[28] It appears obvious that the argument is a part of two larger debates, namely, the role and importance of the autopsy in general, and the controversy regarding the obligations of physicians to provide services to HIV-positive patients.[10,29]

THE ROLE OF THE PATHOLOGIST

What should be the role of the pathologist and the organized pathology community in response to the threat of transmission of blood-borne disease? I believe that our responses should arise from our traditional roles as providers of patient care services, teachers, and scientists.

The OSHA proposed rule will impose a significant new set of regulations and burdens on clinical laboratories. The responsible laboratory community will want to fulfill its obligations to protect all laboratory workers but will also want to play a role in the development of reasonable and "possible" requirements. In announcing the pending standards for blood-borne pathogens, then Labor Secretary Ann McLaughlin said that it marked the first time OSHA had entered into the "disease business" (*The Washington Times*. January 10, 1989). In view of the inexperience of OSHA and OSHA inspectors in the healthcare and laboratory arena, and instances of regulatory and enforcement "overkill" that have been reported by many pathologists, it will be of critical importance to encourage a final rule that has some flexibility.

Pathologists will play a lead role in the development of institutional programs to protect healthcare workers and to comply with OSHA and other federal, state, and local regulations. In many instances, the pathologist will be the individual who is most familiar with the relevant scientific and epidemiologic information and will be called on to assist in the implementation of OSHA guidelines, universal precautions, and educational programs in the laboratory and the hospital.

The pathologist may be placed in an anomalous position as a result of these responsibilities and the multiple roles he or she may have in most healthcare facilities, roles that may include that of employee, employer, or consulting or "contractual" physician.

Parenthetically, some pathologists have reported that they have been excluded from negotiations between OSHA and the hospital (the employer) because the pathologist was not a hospital employee but had supervisory responsibility for laboratory operations as a result of some other contractual arrangement.

In the hospital setting, pathologists will almost always be members of the medical staff with corresponding duties, obligations, and privileges. The responsibility to advise the medical staff concerning appropriate responses to the threat of blood-borne disease may fall to the pathologists and they may need to assume, by example and by direction, a leadership position in defining medical-staff policies.

Pathologists are now presented with the responsibility to integrate a new and demanding set of federal regulations into the clinical laboratory, a setting that is already beset by considerable resource and fiscal constraints. Compliance with the process of integration will be monitored by an agency of the federal government with little or no prior experience in the clinical laboratory or the hospital environment, and which, by virtue of its political origins, may assume a regulatory stance that will be seen by hospitals and laboratory management as harsh and confrontational.

It will be the difficult task of pathologists to respond in a calm manner, emphasizing at all times what we know to be the salient scientific, epidemiologic, and clinical facts. We need to continually assert the importance of education of our coworkers, the rational application of technology, the flexible implementation of precautions based on an understanding of the tasks being performed, and above all our dedication to our own safety and that of our coworkers and our patients.

I am grateful to Harold S. Kaplan, MD, for introducing me to the discipline of risk assessment.

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The Prevalence of Markers for Transfusion-Transmitted Disease in Donations From Different Groups of Blood Donors
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D. P. Drotman; R. O. Valdiserri (*JAMA.* 1989;262:3465)

Applying the Brakes to the Runaway American Health Care System
N. E. Savies; L. H. Felder (*JAMA.* 1990;263:73)

The Autopsy in Acquired Immunodeficiency Syndrome

How and Why

Stephen A. Geller, MD

• The autopsy on the patient who has died of acquired immunodeficiency syndrome (AIDS) can be of great value to the physicians who cared for the patient and to society in general. Although the AIDS virus has been shown to be significantly contagious in the clinical setting, there is no evidence thus far that it is particularly contagious in the autopsy room. Indeed, there is no documentation of a pathologist or autopsy room assistant having contracted AIDS or having seroconverted because of the performance of an autopsy on a patient with AIDS. Procedures to follow in the autopsy of the patient with AIDS are described. The hospital pathologist can safely examine the patient who has died of AIDS by using the well-established techniques for performance of the autopsy.

(*Arch Pathol Lab Med.* 1990; 114:324-329)

The words *hic locus est ubi mors gaudet succurrere vitae* ("this is the place where death delights to help the living") adorn the walls of many autopsy rooms throughout the world and have done so for hundreds of years, since they were first carved into the marble of the great anatomic amphitheater at the University of Padua, Italy. For two centuries, since Morgagni firmly and irrevocably established the principle that the structural bases for clinical events and for death could be found by the physician performing the autopsy, there have been countless contributions to the well-being of mankind emanating from the intellectual exercises carried out in the autopsy room. The many discoveries cannot be recounted herein, for they are literally the textbook of medicine.

Now the tradition of searching for more knowledge about human diseases is seriously threatened by the fear that the autopsy itself may cause serious illness, and even death, for the pathologist.

The autopsy room has always been a potential source for infection, long before the concept of bacteria had been developed. Indeed, it may very well be that the death of his colleague, Prof Kolletschka, the much-admired teacher of forensic pathology at the University of Vienna, Austria, was the critical factor that helped Semmelweiss recognize the possibility of contagion from the autopsy. Kolletschka, one of Rokitansky's two assistants, sustained an accidental laceration during an autopsy and died of an overwhelming wound infection with subsequent sepsis.[1] Semmelweiss recognized the similarity between Kolletschka's fatal illness and those of women with "puerperal sepsis," and this observation led him to the development of his seminal contribution, which, in turn, brought us closer to understanding the nature of contagion.

How many of us have cut our hands at the autopsy table, just as Kolletschka did? How many nicks? How many punctures? Organisms that we no longer recognize as dangerous to the pathologist did, at one time, cause a variety of infections, and even death, among those pioneer physicians who studied their patients who had died. Our professional ancestors worked without ventilated rooms, without easily cleaned stainless steel tables, without disinfectants, and without the ready protection of antibiotics. Today, pathologists are not especially concerned about the usual pyogenic organisms. Pathologists are not even particularly concerned about tuberculosis, although we probably should be.

Where would medicine be today if Bichat, Laennec, Baillie, and Rokitansky, among countless others, had declined to perform autopsies on patients with tuberculosis? Is the actual risk of acquiring a serious illness in the autopsy room greater today than it was a century ago?

Tuberculosis has always been a potential risk for the pathologist and remains so, although the incidence of the disease is relatively low. There is growing evidence, however, that tuberculosis is increasing in incidence, and the pathologist must remain wary.

Creutzfeldt-Jakob disease is also regarded as a risk for the pathologist, although, unlike tuberculosis, there is no documentation of transmission of the disease to a prosector, even before the time when the extraordinary durability of the causative virus became known.[2,3] There are probably as few as 200 cases of Creutzfeldt-Jakob disease in the United States each year, and the likelihood of contact with any pathologist is extraordinarily small. Recommendations for precautions to take for suspected cases have been reviewed.[4]

All patients who die and come to autopsy must be considered as potential sources of infection with hepatitis virus. Indeed, the single greatest risk to pathologists and ancillary personnel is viral hepatitis, both hepatitis B and non-A, non-B (NANB) hepatitis.[5] Hepatitis B can be tested for, and medical

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personnel can, and should, avail themselves of hepatitis B vaccine. There is no vaccine for NANB hepatitis, although one could be developed in the coming years now that at least one form of NANB hepatitis has been identified.[6]

The issue today, of course, is acquired immunodeficiency syndrome (AIDS). Few issues in pathology, in modern times, have contributed to as much concern and disagreement as the question of whether or not autopsy should be performed on the patient who has died of AIDS. This issue has transcended pathology in its direct effects, as increasing numbers of clinicians obtain permission for autopsy on their patients who have died of AIDS, only to be advised that the pathologist is not willing to participate in the postmortem examination.

This article will discuss some aspects of the AIDS virus that pertain to these issues, will review the evidence concerning risk to the pathologist and ancillary staff in performing the autopsy on the patient with AIDS, and will comment on the reasons for autopsy in this population group. There will also be a review of procedures to follow during the performance of the autopsy, based on recommendations of the Centers for Disease Control,[7,8] the National Committee for Clinical Laboratory Standards,[9] and the guidelines promulgated by the Office of Health Compliance Assistance of the Occupational Safety and Health Administration (OSHA).[10] My personal views of the need for, and practicality of, implementation of the various recommendations will be included and will be identified as opinion. Finally, I will address the issue of whether or not it is acceptable for the pathologist to refuse to perform an autopsy.

THE AIDS VIRUS

Human immunodeficiency virus (HIV) is now recognized as the causative agent for the development of AIDS.[11,12] It is a human retrovirus, related to human T-cell lymphotropic virus type I. Both are tropic for the CD4 molecule, and consequently, $CD4^+$ T-cells are principal targets for infection. Unlike human T-cell lymphotropic virus type I, which is a transforming virus with a high propensity for development of neoplasia,[13] HIV is cytopathic, leading to lysis of T cells and subsequent profound immunodeficiency.

Human immunodeficiency virus is spherical, with a lipid membrane derived from host-cell membrane during budding of the virus from the infected cell. The lipid membrane surrounds the virion core, which contains several core proteins and two strands of RNA to which are attached the enzyme reverse transcriptase that characterizes all retroviruses.[13] The viral envelope is covered by two viral glycoproteins, gp41 and gp120, which help attach the HIV to the target cell. The HIV genome is approximately 10 kilobases long and contains the *gag* gene for encoding the core proteins, the *pol* gene for encoding reverse transcriptase, and the *env* gene for encoding the envelope proteins, as well as at least five other genes, some of which regulate the pathogenicity of the virus.

The ways in which the virus can be acquired are well known. Initially recognized as a disorder of homosexual men,[14,15] AIDS is now known to be parenterally transmissible, either through blood and blood product transfusion or through the sharing of hypodermic needles by intravenous drug abusers. Heterosexual transmission has been documented but is probably not as prevalent as was once feared. Accidental transmission in the health care setting is also well known but still quite uncommon.[16-23]

How Is the Virus Inactivated?

Human immunodeficiency virus is not particularly hardy. It is readily inactivated by a wide range of disinfectants, including 4% buffered formaldehyde solution, 50% ethyl alcohol, 3% hydrogen peroxide, phenolic compounds, iodophor compounds, and sodium hypochlorite (household bleach) in a freshly prepared 1:10 dilution in water, leading to a final concentration of 0.5%.[24-26] The virus also requires moisture and will not survive prolonged drying.

What Is the Risk to the Pathologist?

A number of reports deal specifically with the issue of transmission to health care workers in the health care setting.[16-23] There is, thus far, no evidence of transmission of HIV, documented by either seroconversion or clinical AIDS, to a pathologist or autopsy room assistant, although other health care workers have been identified as most likely infected in the work setting. This was recently confirmed in a survey of hospital pathology department directors.[27] Although anecdotal, the survey failed to identify a single instance of infection with HIV among autopsy personnel, despite the fact that autopsies have been performed on patients with AIDS for almost a decade in our largest cities, before the potential infectivity of the condition was recognized. It has been well documented that health care workers in whom AIDS develops almost always belong to recognized high-risk groups.[17,28,29] In contrast, there is significant risk to the pathologist of becoming infected with hepatitis B or NANB during the course of an autopsy.[5,30] There is also growing concern, in the clinical setting, that health care workers are at risk for the development of pulmonary tuberculosis, of the usual *Mycobacterium tuberculosis* type.[31] It is certainly reasonable to expect that the pathologist will similarly be at risk as the incidence of this disorder increases. There is still no evidence, however, of significant risk for development of *Mycobacterium avium-intracellulare* or cytomegalovirus, or any of the other opportunistic infections that affect individuals who are no longer immunocompetent and that are usually related to reactivation of a previously acquired latent infection.[32]

WHY DO AUTOPSIES IN AIDS CASES?

Regrettably, increasing numbers of pathologists regard this as a reasonable question and even question the need for autopsies in general. The recent literature dealing with the potential usefulness of the autopsy as an effective approach to continuing education and quality assurance leaves no doubt about the value of the autopsy in the modern practice of medicine.[33-37] There is even cumulative evidence suggesting an association between a high autopsy rate and the quality of medical practice,[38] although a truly scientific model to test this contention reliably has not yet been developed.

The value of the autopsy in the case of patients who have died of AIDS has clearly been shown.[39,40] In a study of 101 adult patients who died of AIDS, 74% had AIDS-related disorders that had not been suspected clinically but were well documented at autopsy. Furthermore, in this same group of patients, the incidence of non-AIDS-related disorders that had not been suspected clinically was higher than in autopsy studies of patients without AIDS.

There is much to be learned from the death from AIDS. Those who have had the opportunity to study this disorder at the autopsy table in a large number of patients recognize that no two such deaths are identical. The specific cause of death is often unsuspected, the many infectious and neoplastic complicating disorders may not have been recognized during life, and the observed clinical manifestations may be reflections of the effects of more than one pathogen or disorder and may be treatable if recognized.[39,40-43] We can expect that increasing experience with this disorder will be accompanied by the development of effective and specific therapies contributing to long-term survival and even cure. The application of better therapies can only be useful if diagnoses are correctly established.[41]

The autopsy is also important for the pathologist who may be called on to diagnose the variety of relatively uncommon conditions that afflict the still-living patients with AIDS. The autopsy is the place to see the full range of lesions and impaired host responses that are unique to this group of patients, and it allows for the development of sufficient familiarity with the morphologic presentations so that diagnoses can be reliably established during life. For example, it has been well demonstrated that the morphologic patterns of Kaposi's sarcoma vary greatly in the patient with AIDS.[44,45] We receive intestinal biopsies in consultation, a few times a year, in which the referring pathologist is unable to establish the diagnosis of Kaposi's sarcoma. That difficulty, we believe, obtains at least partly because the pathologist has not had the opportunity to study autopsy cases in which a range of morphologic patterns for this protean disorder may be seen. Generally, our residents who have studied Kaposi's sarcoma in autopsied patients establish this diagnosis on the consultation case without difficulty. In a single patient, the pathologist could have the opportunity to study pneumocystosis, toxoplasmosis, and cryptosporidiosis, as well as immunoblastic lymphoma or Kaposi's sarcoma.

PROCEDURES TO FOLLOW IN THE AUTOPSY

The most important factor in assuring the well-being of the pathologist and coworkers remains the pathologist. The greatest danger is not the case itself, or the quality of the autopsy room, but the pathologist's lack of regard for potential risk and lack of concern for sensible and thoughtful technique.

Excellent practice habits should be developed and followed in all cases. There are increasing numbers of individuals coming to autopsy with subclinical and even unsuspected HIV infection.[46] Those who believe that the HIV-infected patient can be avoided are deluding themselves, unless they plan to stop doing autopsies altogether. The motorcycle accident victim who is not known to be gay and does not yet have HIV antibodies may be in the serologic "window" period, but his blood may be teeming with viruses. The next myocardial infarction patient may be gay but may not have revealed that history; he may have no evidence of AIDS, may not have been tested for presence of the antibody, but may have an HIV viremia. The elderly woman dead of a cerebrovascular accident may have received a blood transfusion before widespread implementation of blood donor screening, or may have received a unit of blood from a virus-positive patient in whom an antibody response may not have been demonstrable.[47]

All of these individuals, and many others, can have HIV in their blood, as well as hepatitis B or NANB. The only real protection for the pathologist is knowledge and understanding, good autopsy technique, and safe practice.

Is a Special Autopsy Facility Needed?

A number of institutions have elected to perform the autopsy on the case with AIDS, hepatitis, or other known infection in a special "isolation" room.[27] Most do not have access to this kind of facility. We do not believe that this is necessary for AIDS or hepatitis, but it would certainly be appropriate and necessary for the autopsy performed on a patient who has died of a highly communicable disease, such as plague.[48] Again, a facility for known infectious cases will not protect against the increasing numbers of patients who will have HIV but have not been clinically recognized as infected. Certainly, the autopsy room should be clean, easily cleaned, well ventilated, and well lit.

Should the Autopsy Room Be Closed Except for Essential Personnel?

It has been suggested that the autopsy room be closed except for the prosector and assistant.[9] This, of course, would greatly diminish the potential educational value of the autopsy. There is no evidence to support this policy, since there is no convincing evidence that HIV is transmitted in the air.[11] Furthermore, health care workers who seroconvert or develop AIDS do not do so because they have viewed an autopsy.[16-23] Certainly, individuals who are themselves immunosuppressed or immunodeficient should not visit the autopsy room, whether or not there is a known case of AIDS.

In general, the autopsy room should be closed to random traffic but, we believe, should continue to be a place where physicians can learn. It may be appropriate, however, to require visitors to wear at least some form of protective garb.

Are There Any Limitations on Autopsy Personnel Themselves?

Ideally, there should be a prosector and a "clean" circulating assistant. Clearly, immunosuppressed or immunodeficient individuals should not perform autopsies. Furthermore, individuals who have uncovered wounds, weeping skin lesions, or other dermatitides should not participate in the autopsy.

What Protective Garb Should Be Worn?

The recommendation of the National Committee for Clinical Laboratory Standards is for total-body barrier protection with water-repellent protective clothing that includes caps or hoods that completely cover the hair. A plastic face shield is advised to cover the entire face and neck region, or, alternatively, safety goggles with a protective cushion seal. Masks to cover the mouth and nose are listed as well as water-protective boots, double surgical gloves, heavy overgloves, and, for certain procedures, stainless steel mesh gloves.[9] The proposed OSHA regulations[10] indicate that total-body coverage, using fluid-proof materials, will be required. The OSHA regulations, of course, carry with them the potential for monetarily punitive actions. Total-body disposable suits that include total head enclosure and foot cover ("space suits") are commercially available.

We have no objection to this type of coverage, and do make "space suits" available for our residents and staff, but we do not believe they are necessary. Forearms can be protected by wearing waterproof forearm guards, of the kind used in the operating rooms. These are also not necessary if the prosector dissects carefully. The performance of the autopsy should not be accompanied by the splashing about of blood and body fluids. Prosectors' skin surfaces that do become contaminated should be promptly cleaned. Furthermore, the commercially available waterproof covering garments, including the total-body space suits, can become sufficiently soaked so that they become permeable and do not necessarily protect against poor technique.

Although there is no convincing evidence indicating the need for face masks in performing the autopsy, we support their use, as well as the use of effective eye protection. Recently developed face masks do include an eye shield, but the shield is not protective of the sides of the head and the lateral corners of the eyes. Regular glasses are not adequate protection, and, at a minimum, wraparound goggles should be employed.

Regular surgical gloves may be permeable because of manufacturing defects or may become permeable quickly with use.[49] For this reason, a number of pathologists choose to double glove. This may, in itself, be dangerous if the gloves do not fit tightly. One of the risks of double gloves is that sensation may be slightly diminished, particularly if the fingertip portion of the outer glove is slightly loose, inviting accidental cuts. Certainly, both pairs of gloves may become porous after relatively short periods of usage.[50] There is no evidence supporting the view that single-glove use is dangerous. In either case, gloves should be changed promptly when there is seepage under the glove or any obvious interruption in the integrity of the glove. We recommend that gloves be frequently changed, whether or not there is obvious evidence of tears or leakage.

Heavy gloves made of latex, neoprene, or other plastics can be used. These are somewhat clumsy to use and could potentially be dangerous if not used carefully, since it is easy to drop instruments. Latex gloves, of the kind that are available in the supermarket, are slippery when wet, and it may be difficult to hold instruments. Variants, available from laboratory supply houses, have grooved surfaces at the fingertips that improve their potential for holding.

Stainless steel gloves can be obtained, made up of coarse chain mail or finely woven stainless steel fabric. The chain mail gloves are, in our experience, quite difficult to use. They do have a role, we believe, in special settings where there is particular risk of cutting oneself, such as in the removal of pelvic contents. In this setting, we recommend the use of the stainless steel mesh glove to grab the pelvic organs and retract them, while severing the attachments with a long knife.

It is especially difficult to hold instruments with the chain mail glove unless a latex glove is worn over the metal. The fine woven steel gloves are considerably more flexible and, for those who seek the protection of metal, are certainly easy to use. It is important to remember that none of the gloves currently available will prevent penetration by a needle. Furthermore, although the finely woven steel glove will resist the usual type of cutting, it will not prevent a forceful stab wound.

Should Special Instruments Be Used?

There is no need for special instruments. The pathologist may want to select materials that are autoclavable. At a minimum, they must be cleaned in disinfectant such as sodium hypochlorite (bleach). Aluminum and stainless steel are damaged by sodium hypochlorite, and instruments made of those materials should be decontaminated with a 2% aqueous glutaraldehyde solution. In the rare case of Creutzfeldt-Jakob disease, prolonged soaking in sodium hydroxide solution is recommended.[4]

Should Many Scalpel Blades Be Prepared So That Blades Do Not Have to Be Changed?

There should only be one set of sharps. Some pathologists prefer to have many scalpel blades available, so that they do not have to change blades in midautopsy. A dull blade is a dangerous blade, since cutting with a dull blade requires more force than with a sharp blade. Pathologists should learn how to remove scalpel blades. The blades most widely used were designed to be handled by the operating room nurse so that they could be easily and safely changed without special devices. There is a dull area on the bottom to lift the blade slightly from the handle, and a ribbed area at the top to slide it on or off the handle.

Simple wooden blade removal devices are available, however, if the pathologists wants to keep hands completely away from the blade. A forceps or a surgical clamp should *not* be used. These often lead to "flying blades" and can contribute to serious accidents.

Blades should not be passed from one prosector to another. They should be put down on a firm, stable surface by one prosector and picked up from that surface by the second. In general, a nonpointed scissors should be used wherever possible.

What Do We Do About Cutting Bone?

The ribs should not be cut with an oscillating saw. This contributes to jagged, bony rib edges that can scratch or even lacerate. The ribs should be cut through the cartilaginous portion, immediately medial to the costochondral junction. The cartilage is, of course, soft and will not contribute to accidental injuries.

In most patients, unless they are quite old, the clavicle and sternum can be separated with a scalpel. The first rib, however, will have to be severed with a rib cutter.

The oscillating electric saw (Stryker) should be used sparingly but can be used to cut other bones as needed. There is considerable aerosolization with the oscillating saw. Although a vacuum attachment has been recommended,[9] it really serves little purpose since it does not significantly reduce aerosolization. There is no evidence that HIV is spread by aerosolization,[11] but tuberculosis certainly can be, and efforts directed at reducing aerosolization are certainly recommended.

Many techniques have been recommended to reduce the aerosolization that follows removal of the vertebral bodies or opening of the calvarium, including attempting to contain the effect with a large plastic bag or shielding the spray by keeping wet towels immediately adjacent to the blade. These approaches are also less than completely effective and are difficult to employ. Recently, a number of devices have been designed that fit on the autopsy table, prevent aerosolization into the environment, and allow a fair amount of flexibility.

Should All Organs Be Fixed?

The pathologist should fix all organs in a known infective case. Although the AIDS virus is not easily recovered at the time of autopsy from the patient who has died of AIDS, patients may also harbor hepatitis virus, which must be inactivated. There should be at least 10 parts of 4% neutral buffered formaldehyde solution for each part tissue. Formaldehyde solution will not penetrate more than 4 to 5 mm in 24 hours, and tissue slabs should be no greater than 2 cm thick and should be separated by towels or papers. Bloody formaldehyde solution is not an effective fixative and should be changed.

The OSHA regulations require that all formaldehyde solution containers be appropriately labeled, even fixing pans. Unfixed organs can be placed in a plastic bag and returned to the body cavity at the end of the autopsy, or incinerated. When fixed tissues have served their purpose, they should be incinerated.

Should Fresh Organs Be Photographed?

Clearly, the educational value to be obtained from a photograph of macroscopic disease is greater when the organ is fresh. Discretion with blood and body fluids is appropriate, and photographic stands and cameras should not be contaminated. The HIV virus will not survive for long periods with drying,[24-26] but the tubercle bacillus certainly will. The photographic area should be kept scrupulously clean, and organs should be transported to the camera area with careful attention to avoiding spills. Obviously, gloves used to handle specimens should not be used to manipulate the camera.

Can an Autopsy Be Performed Safely on Patients With AIDS in the Average Hospital Without Risk to the Community?

There is absolutely no evidence that hospital waste has caused disease in the community when it is handled with the usual disposal methods.[7] It is entirely reasonable, however, to identify wastes with the special potential for causing infection and develop methods of handling that are practical and effective. Infectious waste, in general, should be incinerated or autoclaved before disposal in a sanitary landfill.[9,11] Fluids, including blood, may be poured down a drain connected to a sanitary sewer if allowed by local health codes.

Are Special Procedures Required for Preparing the Body After Autopsy?

The closed body should be carefully washed with a detergent solution, then with dilute sodium hypochlorite solution, and finally rinsed with water. If there is a known contagious organism, the body bag should be specially marked. This, of course, is not exactly according to the precepts of the universal precautions. There is evidence that specially marking blood tubes reduces the level of concern about, and vigilance for, unmarked tubes.[51] This could increase infection transmission. There are no data about the advantages or disadvantages of designating potential infectivity of bodies, but it is probably advisable as a warning to morticians and related staff. Although the risk of AIDS in this circumstance certainly is virtually nil, the risk of hepatitis is significant.

How Should the Room Be Cleaned?

The table, instruments, floors, and other stable structures should be carefully washed with a detergent to remove the dried blood and fluids. Then the detergent should be rinsed away and the structures should again be washed with a 1:10 dilution of household bleach or other germicide, rinsed again, and dried. This procedure should be followed in every case, and should include the body-holding area and the refrigerator. For the exceedingly rare case of Creutzfeldt-Jakob disease, surfaces and instruments should be washed with sodium hydroxide solution.[4]

How Does the Pathologist Clean Up?

Ideally, there should be a shower adjacent to the autopsy room. In many institutions, this does not exist. At a minimum, autopsy personnel should discard all disposable materials, gowns, shoe covers, gloves, and other items in red-marked bags, and the reusable materials in other containers for autoclaving. Adequate washing facilities must be available in the vicinity of the autopsy room.

This discussion has not touched on all details of performing the autopsy, and additional sources[7-11] should be referred to when developing a laboratory policy. The most important factor in assuring the well-being of the pathologist and coworkers remains the pathologist. Sensible and aesthetic discretion with blood, feces, body fluids, and tissues will contribute greatly to overall safety. The greatest danger in most settings is not the patient or the autopsy facility, but the pathologist's lack of regard for the potential risk inherent in every autopsy. The classic techniques of the autopsy, which were developed when tuberculosis was greatly feared, need to be reintroduced into residency programs and into the practice of pathology.

CAN THE PATHOLOGIST REFUSE TO PERFORM AN AUTOPSY ON THE PATIENT WITH AIDS?

There is no justification for the pathologist to refuse to perform an autopsy because the patient has been infected with HIV, particularly since there is no evidence that careful autopsy technique, as traditionally practiced, places the pathologist at risk for acquiring that infection. Furthermore, there is no justification for the pathologist to refuse to perform an autopsy on the premise that there may be little to learn from a given autopsy, since it has been well documented that it is not possible to predict whether or not an autopsy will yield clinically unsuspected findings.[38,39] The only reasonable justification, in my opinion, for refusing to perform a postmortem examination in the case of the patient who has died with documented or suspected HIV infection is when the limitations on performance of the autopsy, as listed on the form authorizing autopsy, are so severe that they will prevent the pathologist from meeting the obligations to the profession. The ethical requirements for the pathologist, in terms of AIDS, are in no way different from those for other physicians.[52-54]

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Current Risk of Transfusion-Associated Human Immunodeficiency Virus Infection

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• The procedures designed to minimize the risk of human immunodeficiency virus (HIV) transmission through transfusion include donor self-exclusion, health history questions, confidential unit exclusion, donor call back to withdraw donation, and HIV antibody testing. Each step is important for reducing the number of units collected from donors who are at risk for HIV infection. Although HIV antibody test kit sensitivity exceeds 99%, recently infected persons who are in the "window" between infection and seroconversion are not detected. "Worst scenario" estimates indicate that 1 of 36 000 to 1 of 300 000 components may be collected from donors who have false-negative test results. Since some risk of infection transmission remains, physicians must prescribe transfusion therapy only when the benefit outweighs the potential risk.

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Blood transfusion is a therapeutic intervention in which viable cells not subjected to viral inactivation procedures are transferred from one person to another. To reduce the likelihood of transmitting infection by transfusion, donors undergo a multistep screening process. Specifically with regard to human immunodeficiency virus (HIV) infection, donors receive information about activities placing people at risk for HIV infection, are asked medical history questions pertaining to acquired immunodeficiency syndrome (AIDS), are instructed to use the confidential unit exclusion (CUE) procedure, and have a sample of blood tested for antibody against HIV. In addition, donors may call back to the organization collecting their blood if, after donation, they acknowledge their previous participation in activities placing them at risk for HIV infection. Individually, these measures are effective. Cumulatively, they provide an effective mechanism for safeguarding the blood supply. Nevertheless, in the absence of an effective viral inactivation procedure, the risk is not, and will never be, zero. Currently, "worst scenario" estimates indicate the risk of contracting AIDS through a blood transfusion is approximately 0.0025% to 0.0003% per component.

SCREENING MEASURES

Self-exclusion

Following the reports of *Pneumocystis carinii* pneumonia in hemophilic patients and AIDS in an infant who received a platelet concentrate donated by a person who subsequently developed AIDS, major US blood-banking organizations issued guidelines in January 1983 for reducing the risk of acquiring AIDS through transfusion.[1-3] The Food and Drug Administration (FDA) made similar recommendations to blood collection establishments in March 1983.[4] People with symptoms of AIDS, homosexually active men with multiple sex partners, intravenous drug abusers, hemophilic patients, immigrants from Haiti, and sexual partners of persons at increased risk for AIDS were asked not to donate because they might be at risk of transmitting AIDS to a transfusion recipient. These recommendations were made despite a lack of evidence documenting transmission of AIDS through transfusion.

Since the appropriate response by persons at risk for HIV was to defer themselves and not present for blood donation, it is impossible to quantitate the success of this approach. However, in the greater New York City, NY, metropolitan area, during comparable 9-week periods in 1982 and 1983, the percentage of male donors decreased from 62.6% to 61.5% ($P<.001$).[5] The most significant decrease occurred in New York City where 12% fewer males between the ages of 21 and 35 years donated blood during the study period in 1983 compared with the same time in 1982 ($P<.001$). The age and geographic distribution of the donors no longer presenting at blood collection sites indicated that many homosexually active men had voluntarily stopped donating blood in New York.

A retrospective analysis of the donation practices of persons who developed AIDS in the San Francisco, Calif, area also supports the effectiveness of the self-exclusion process. The names of persons with AIDS in San Francisco were compared with the donor

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files of the Irwin Memorial Blood Bank in San Francisco. One hundred ninety-nine persons made 634 donations before they developed AIDS. Most of these donors ceased donating blood in 1983, indicating that self-exclusion policies were effective.[6]

Further evidence supporting the effectiveness of self-deferral relates to changes recorded following modification of the informational message presented to donors. For example, prior to October 1985, "males who have had sex with more than one male since 1979, and males whose male partner has had sex with more than one male since 1979," were listed as a risk group for AIDS. The wording was changed to "a male who has had sex with another male anytime since 1977." Subsequently, the number of donors found to be positive for HIV antibody decreased from 0.033% to 0.0084% in Connecticut. This change was attributed, in part, to the revised donor information material.[7]

Although effective, the self-exclusion process did not result in total cessation of at-risk persons from donating.[8] Approximately 1 of 10000 donors nationwide continues to be found positive for HIV antibody. The majority have a history of high-risk activity.[9] They donated because they had few or no recent risk exposures, were in good health, did not consider themselves to be HIV carriers, felt pressure to donate at the work site, or donated to get HIV testing.[10,11]

Donor History Questions

The blood donor history questions were changed in 1983 to determine whether donors had signs or symptoms of AIDS, fever, night sweats, thrush, lymphadenopathy, or Kaposi's sarcoma. Approximately 0.4% of potential donors were rejected by the Greater New York Blood Program in 1983[5] because of affirmative answers to these questions. The questions were designed to detect donors with AIDS who did not self-defer and also to reinforce concern about AIDS among asymptomatic donors.

After routine HIV antibody testing was implemented, the need to ask these questions was reexamined, since it was expected that persons with AIDS-related symptoms would be positive for HIV antibody. Concern that deletion of these questions would indicate blood collection agencies were lowering their vigilance about donor screening has, in part, resulted in the continuation of the use of these questions.

CUE Method

Confidential unit exclusion is a confidential (but not anonymous) method for donors to indicate their blood donation should not be used for transfusion to patients. This CUE is designed to prevent the blood from those persons at risk for AIDS who perceive peer pressure to donate from entering the general blood supply. Initially, CUE was implemented at the Greater New York Blood Program by asking donors to complete a confidential form that described AIDS high-risk groups and the possibility of AIDS transmission by blood transfusion.[5] The donor was then requested to indicate whether "my blood donation should be used only for studies" or "my blood donation may be used for transfusion." Donors completed the form at privacy stations. The form contained the identifying number attached to the blood unit but did not have the donor's name or signature. Standard tests were performed on all donated units, and donors with abnormal results were notified of such results. Overall, 1.4% of Greater New York Blood Program donors excluded themselves. An additional 1.6% did not complete the questionnaire.

In New York City, 70% of donors who excluded themselves were men compared with 62.5% among the "for transfusion" donors ($P<.001$). Male donors less than 36 years of age had a self-exclusion rate of 1.6% compared with 0.8% for those older than 36 years ($P<.001$). The prevalence of test results positive for hepatitis B surface antigen was 0.49% among those excluding units compared with 0.27% in donors signifying their blood could be used for transfusion. Of donors excluding themselves, 11.9% were positive for hepatitis B core antigen compared with 6% of donors who had designated their blood "for transfusion." Concern arose that self-exclusion at the time of donation would serve as a stimulus to members of high-risk groups to provide blood for studies. It was thought that this did not occur. Overall, investigators at the New York Blood Center thought CUE added to the other measures used to decrease the risk of transfusion-associated AIDS. The FDA issued a memorandum recommending the use of CUE in October 1986.

The postulated benefit of CUE is supported by a series of studies performed at the Toronto Center, Canadian Red Cross Blood Transfusion Service.[12-14] Donors who designated their blood for laboratory purposes were found to have higher rates of confirmed positive tests for HIV antibodies and other viral markers than those who indicated their blood could be used for transfusion.

The consistency with which donors used CUE was studied in Toronto. More than 99% of donors who indicated their units were safe for transfusion made the same designation at the time of their next blood donation. However, approximately one third of donors who designated their blood to be used for laboratory studies only at the time of one donation gave the same response at subsequent donations. In addition, donors indicating their blood should be used for laboratory purposes only tended not to return to donate as often as those who indicated their blood was safe for transfusion.

The efficacy of CUE has been questioned.[15-17] A perceived lack of benefit may be related to the effectiveness of the donor educational materials and the donor's understanding of the system. A decrease in the number of donors selecting the option indicating laboratory use only for their blood was observed among persons donating in southeastern Wisconsin when the CUE instructional format was modified to include a pictorial description (J.E.M., unpublished data, 1989). This was interpreted to mean that the instructions initially used were not optimal.

Call Back

The American Association of Blood Banks, American Red Cross, and the Council of Community Blood Centers issued a recommendation in January 1984 that blood collection agencies implement a system by which persons in high-risk groups for HIV infection who were donating (presumably because of peer pressure to do so) could "call back" to have their unit removed from the general blood inventory supply. The efficacy of this approach has been difficult to document, although there are anecdotal reports of donors using this system. Following introduction of CUE, the call-back system was believed by many to be superfluous. However, it is used in conjunction with CUE at some centers.

HIV Antibody Testing

The discovery of HIV, the etiologic agent for AIDS, was announced in April 1984.[18] The FDA approved license applications for test kits in March 1985. During the spring of 1985, HIV antibody testing of blood donors became routine. Approximately 0.04% of donors had confirmed positive results.[19] Blood collected from donors who are positive for HIV antibody is destroyed, and the donors are notified, counseled, and deferred from further blood donation.

Test introduction did not obviate the need for the other donor screening measures, however. The tests detect antibody, and hence, some recently infected persons (who have the potential to transmit HIV) might have negative test results.

False-positive HIV Antibody Test Results.—The specificity of HIV antibody test kits also poses important questions. Test kits with a high degree of nonspecificity create a cohort of donors with false-positive results, ie, donors with repeatedly reactive enzyme-linked immunosorbent assay (ELISA) test results that are not confirmed by more specific testing such as Western blot or radioimmunoprecipitation assays. The causes of nonspecific reactions include cross-reactivity with HLA antigens present in the cell lines used for preparing antibody test kit reagents, cross-reactivity with other retrovirus infections (eg, bovine retroviruses), or unknown reasons.[20-23] A small percentage of donors with indeterminate Western blot test results (P24 reactivity only) have been shown to have complete seroconversion patterns when tested subsequently.[23,24]

In general, distinguishing those with false-positive results from those in the early stage of seroconversion is not always possible on the basis of testing a single sample. For this reason, all donations from persons with repeatedly reactive ELISA results are not used for transfusion purposes. Currently, the FDA allows "reentry" of donors with repeatedly reactive ELISA results and negative confirmatory test results if a specimen obtained at least 6 months subsequently is nonreactive on both ELISA and Western blot tests. The time interval between the first and repeated specimen collection is considered sufficient for a person to evolve from an indeterminate seroconversion pattern to one that would be reactive unequivocally. Hence, truly infected individuals would test positive while those with nonspecific reactions would be clarified as having false-positive results. Subsequent donations are entered into the general blood supply.

False-negative HIV Antibody Test Results.—False-negative results are defined as those in which the ELISA test for HIV antibody is negative despite the test subject's presumed ability to transmit HIV. These persons are identified retrospectively when their blood transmits HIV despite a negative HIV antibody test result. In addition, techniques that are more sensitive than antibody testing, such as those using gene amplification procedures, have demonstrated the presence of HIV in these donors despite negative serologic findings.

Window Phase HIV Antibody Testing.—The interval between infection and the time of seroconversion (the so-called window) is thought to be 2 to 6 months.[25] However, several reports indicate persons may be infected for prolonged periods prior to seroconversion.

Ranki et al[26] detected free HIV antigen and/or low titer antibodies to structural or nonstructural HIV proteins 6 to 14 months before seroconversion in nine persons who seroconverted as determined by routine techniques. Loche and Mach[27] analyzed blood samples from 16 seronegative sexual partners of HIV-infected homosexually active men by polymerase chain reaction (PCR) techniques. Five were positive for HIV DNA during a 3-month period when the serologic results were negative.

Recently, Imagawa et al[28] reported HIV was isolated from 31 (23%) of 133 men by viral culture technique, 27 of whom were seronegative for up to 35 months after the positive culture. Four seroconverted 11 to 17 months after the positive HIV culture result, and 3 of these patients, who had cryopreserved lymphocytes, had evidence of proviral DNA by PCR. The provirus was detected 23 to 35 months before seroconversion. The authors concluded that a prolonged period of latency may be more common than previously recognized.

Evidence that does not support the presence of a prolonged latent period has been presented by Groopman et al.[29] They conducted a longitudinal study of sexual partners of patients with AIDS or AIDS-related complex. No serologic or viral culture evidence of HIV infection was found in serum samples obtained up to 24 months prior to the time of seroconversion. Also, Jackson et al[30] used PCR techniques to study heterosexual partners of HIV-infected hemophilic patients who practiced unprotected sex. They found no evidence of HIV infection in persons whose test results were negative for HIV antibody.

It is hoped the controversy surrounding the length of the window period and the frequency with which HIV transmission occurs during this period will be resolved. Until this occurs, most decision-makers consider 6 months as the practical outside limit during which seroconversion occurs.

Seroreversion.—Farzadegan et al[31] reported that 4 of 1000 men involved in the longitudinal Multicenter AIDS Cohort Study reverted from seropositive to seronegative for HIV antibody over a 2½-year period. The PCR assays done on peripheral blood mononuclear cells when the ELISA tests were negative showed HIV provirus in the 4 men 6 to 18 months after the last positive antibody test result was obtained. They concluded the incidence of seroreversion is low (0.4%). Nevertheless, the authors were unable to demonstrate that the men continued to be infectious to others.

Nonspecific Testing.—Prior to the availability of a specific marker for HIV infection, the use of nonspecific or surrogate tests was advocated by some. The intent was to test for markers found in a higher percentage of persons in AIDS risk groups than in the general blood donor population. By doing this, it was hoped that a laboratory test abnormality would identify those at risk of transmitting AIDS through transfusion. Several studies were performed to determine the efficacy of using such nonspecific tests as the hepatitis B core antigen test, T-helper/T-suppressor lymphocyte ratios, and circulating immune complexes. Several of these tests were impractical to implement in blood centers. The test for hepatitis B core antigen, although positive more frequently among patients with AIDS than blood donors, did not identify those who might transmit AIDS.[32] Overall, the ability of surrogate tests to discriminate potentially infectious donors from noninfected donors was not deemed sufficient to justify their implementation on a wide-scale basis.

NARROWING THE WINDOW

The window phase may be narrowed by further refinements in donor history questioning and provision of information, through the use of more sensitive laboratory tests, such as tests for viral nucleic acid and HIV antigen, or by a combination of these approaches.

Use of Specific Questions About Risk Behaviors

In 1983, the American Association of Blood Banks, American Red Cross, and Council of Community Blood Centers issued a statement indicating that

> direct or indirect questions about a donor's sexual preference are inappropriate. Such an invasion of privacy can be justified only if it demonstrates a clear-cut benefit. In fact, there is reason to believe that such questions, no matter how well intentioned, are ineffective in eliminating those donors who may carry AIDS.

More recently, investigators in southwest Florida and San Bernardino, Calif, conducted a pilot study to determine whether more specific questions asked orally would insult donors and elicit angry responses or whether they would be helpful potentially in identifying some individuals who had participated in AIDS risk factor activities.[33] They found the questions were acceptable, in general, to donors. Moreover, there was a suggestion that the questions helped to identify a few persons who participated in HIV risk activities but had failed to self-defer.

In August 1989, the American Association of Blood Banks issued a statement recommending a change in the previous guideline and suggested that all donors receive orally presented information to assure they understand the activities that place people at risk for HIV infection and how to use the CUE process.

HIV Antigen Testing

A brief interval in which P24 antigen is detected by serologic techniques occurs prior to development of HIV antibodies.[34-36] Serum specimens obtained before HIV seroconversion occurred in hemophilic patients and serum specimens obtained from newly infected persons who were symptomatic were negative for HIV antibody but positive for HIV antigen.[34-36] In addition, plasma samples from source plasma donors whose units tested negative by ELISA were retrieved and tested for HIV antigen when subsequent donations were found to be positive for HIV antibody.[37] The HIV antigen was detected in such donors when they were negative for HIV antibody. Although these findings suggest HIV is present in source plasma pools used for fractionation and production of derivatives, the impact on the safety of plasma derivatives is less clear. Albumin and coagulation factor concentrates undergo viral inactivation procedures that are considered sufficient to destroy trace contamination with HIV. Immune globulin preparations undergo fractionation and inactivation procedures that negate transmission of HIV.

On the other hand, whole blood and the components derived from whole blood donations do not undergo viral inactivation procedures. Implementation of HIV antigen testing is postulated as a mechanism for narrowing the window phase. However, the theoretic utility of testing whole-blood donors for HIV antigen was not demonstrated when applied to the routine donor setting. Among 600 000 persons donating blood in Bavaria and Austria, none was found to be positive for HIV antigen and negative for HIV antibody.[38] In a study sponsored by the American Association of Blood Banks and the FDA, none of 500 000 US blood donors was found to be positive for HIV antigen and negative for HIV antibody. This information, in part, led to the FDA's decision not to recommend HIV antigen testing as a screening test for volunteer whole-blood donors at the meeting of the Blood Products Advisory Committee on March 23, 1989.

PCR Techniques

At the current time, the PCR technique is reserved for research purposes for detection of HIV.[39] Development of this procedure is being monitored. If sufficient progress is made, PCR testing will likely be adopted readily by blood collection agencies.

Use of Antibody Tests Using Recombinant Antigens or Semisynthetic Polypeptides

One test kit that uses HIV recombinant antigens to detect HIV antibody is licensed by the FDA. It is recommended for use in emergency situations or when ELISA tests are not available. It has not been recommended for routine blood donor screening.

Previously, the FDA was reluctant to license tests using HIV-derived recombinant antigens for routine blood screening because of the possibility of genetic drift of the HIV virus and the resultant production of antibodies that would not be directed against epitopes contained in the recombinant-derived tests. Currently, this is addressed by choosing antigens derived by recombinant techniques or those chemically produced with semisynthetic peptides that correspond to relatively conserved regions of the virus.

ESTIMATE OF CURRENT RISK

Four published studies and one presented in abstract form indicate the risk of contracting AIDS from donors tested for HIV antibody is approximately 1 in 36 000 to 1 in 300 000 per component transfused.[11,40-43] By contrast, the per-component risk of contracting fatal hemolytic transfusion reaction is 1 in 100 000, and the risk of contracting non-A, non-B hepatitis is 1 in 100 per component.[44] The statistical basis on which these estimates are made is derived mainly from donations collected between March 1985 and December 1987. This corresponds to the period in which the prevalence of donors positive for HIV antibody was higher than that currently observed.[45] Since the rate of donors testing positive decreased at a rate of 30% per year between 1985 and 1987 and has continued to decrease, a recent estimate for the false-negative results as 1 in 153 000 units is more realistic.[42]

ZERO-RISK BLOOD SUPPLY

A prevailing perception is that the public demands zero risk with regard to blood transfusion and the possible transmission of HIV.[46] Unfortunately, zero risk cannot be achieved. This pertains to all therapeutic modalities including blood transfusion. The potential lifesaving benefits of transfusion should not be denied because of anxiety disproportionate to the risk. The risk of acquiring AIDS through a transfusion should be compared with other risks commonly accepted by patients undergoing therapeutic procedures. Ideally, patients should be informed of potential risks and potential benefits associated with transfusion so that an appropriate decision can be made. It is incumbent on physicians to prescribe transfusions only in situations where the benefit outweighs the risk.

CONCLUSIONS

Currently, the risk of acquiring HIV infection through transfusion is approximately 1 in 153 000 per component. This risk pales in comparison with other risks routinely accepted by hospitalized patients. However, it continues to engender emotional discord and outright fear on the part of some patients for whom transfusions are ordered. These patients demand zero risk. Unfortunately, this is not possible. Additionally, it is somewhat ironic that the public continues to focus on this aspect of transfusion when the blood supply is safer than it has ever been. It is hoped the advances made in transfusion safety will continue, and the public will evaluate the risks in perspective.

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Current Safety of Clotting Factor Concentrates

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• Earlier commercial clotting factor concentrates transmitted hepatitis viruses to 100% and acquired immunodeficiency syndrome viruses to 60% to 80% of patients with hemophilia. Transmission of the human immunodefieciency virus was nearly eliminated by heating concentrates in the lyophilized state, which has been done since 1983. However, human immunodeficiency virus infections were still transmitted by some products "dry heated" under conditions less extreme than 68°C for 72 hours. Newer virus-inactivating procedures include "dry heating" at 80°C for 72 hours, modified heating in *n*-heptane or water vapor, heating in solution, treatment with solvent-detergent mixtures, monoclonal affinity purification plus inactivation, and alkylation with beta-propiolactone (only for factor IX complex). These procedures have eliminated significant loads of human immunodeficiency virus, hepatitis B virus, and non-A, non-B hepatitis virus in laboratory studies. However, clinical studies have shown transmission of hepatitis non-A, non-B for products "dry heated" except at 80°C and for products heated in *n*-heptane. Elimination of hepatitis B has been difficult to demonstrate, suggesting a continued need for immunization.

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Although the availability of commercially prepared coagulation factor concentrates, including factor VIII and factor IX complex, has dramatically improved the care of patients with hemophilia A and B since the early 1970s, these plasma-derived products have also transmitted hepatitis B virus (HBV), non-A, non-B hepatitis viruses (NANBHVs), hepatitis delta agent, and human immunodeficiency virus (HIV) to a large percentage of recipients, with significant resultant morbidity and mortality.[1-3] Factor concentrates can also transmit human parvovirus, but infection is probably of no long-term consequence except in persons with preexistent anemia.[4,5] The prevalence of viral markers and the associated diseases in patients with hemophilia are summarized in Table 1. Other bloodborne agents, such as human T-cell lymphotropic virus type I, cytomegalovirus, Epstein-Barr virus, and hepatitis A virus, do not seem to be transmitted by fractionated products. Fortunately for patients not already infected, the introduction of virus-inactivating procedures into the manufacture of coagulation factors, beginning with dry heating in 1983, has virtually eliminated transmission of HIV, and the data suggest that some of the newer inactivation processes, including heating in solution (pasteurization), treatment with solvent-detergent systems, affinity purification plus inactivation, heating in an organic medium, hot vapor treatment, and dry heat under extreme conditions (at least 68°C for 72 hours), have substantially reduced the risk of HIV transmission.[11] Most of these processes also appear to reduce the risks of transmission of HBV and NANBHV.[12-15] Purification by the use of monoclonal antibody affinity not only contributes to viral safety but also may have a further medical benefit in reducing immunostimulation by other contaminating plasma proteins.[15] For some patients, particularly those with von Willebrand's disease, it remains necessary to treat with cryoprecipitate, which is not treated to inactivate viruses. In this instance, it has been recommended that the product be obtained from a single well-screened and repeatedly tested donor or from a small number of such donors whenever possible.[11]

This review will survey the progress that has been made in improving the safety of commercial clotting factor concentrates by summarizing the current methods of inactivation of viruses in these products and the recent studies that have generated a profile of virus killing and clinical safety.

SIGNIFICANCE OF VIRUS TRANSMISSION BY UNTREATED CONCENTRATES

Prior to the use of virus-inactivating steps in manufacturing clotting factor

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Table 1.—Prevalence of Viral Markers in the Hemophilia Population and Their Associated Diseases

Virus*	Marker Prevalence	Associated Diseases
HBV[6]	90% have anti-hepatitis B serum, 5% to 10% have persistent hepatitis B surface antigen	Acute and chronic hepatitis, 10% risk of cirrhosis in chronic cases
NANBHV[7]	25% to 50% have persistently elevated alaninine aminotransferase level	Acute and chronic hepatitis, 20% risk of cirrhosis in chronic cases
Delta agent[8]	50% have antibodies	Acute and chronic hepatitis in HBV-positive subjects, increases severity of hepatitis B
HIV[9]	60% to 80% have antibodies, with 90% in severe cases	Acquired immunodeficiency syndrome and acquired immunodeficiency syndrome-related complex
Parvovirus[10]	88% have antibodies	Acute marrow depression, risk of aplastic anemia

*HBV indicates hepatitis B virus; NANBHV, non-A, non-B hepatitis virus; and HIV, human immunodeficiency virus.

concentrates, all patients treated with over 10000 U of factor VIII concentrate were reported to show evidence of exposure to HBV.[16] Clinical studies have also revealed a rate of transmission of non-A, non-B hepatitis of 30% to 100%.[17] Based on these findings, it is likely that all lots were capable of transmitting these agents. Approximately 20% to 40% of patients developed chronic active hepatitis due either to HBV (10% of patients) or NANBHV, and follow-up studies showed a significant risk of cirrhosis and death,[18] especially for patients with non-A, non-B hepatitis. The impact of HIV has been even more devastating. Since the first recognized cases of transfusion-transmitted acquired immunodeficiency syndrome in 1982, HIV infection has occurred in 80% to 90% of patients with severe hemophilia A and as many as 40% to 50% of patients with mild or moderate hemophilia A or hemophilia B.[19] Fewer patients with von Willebrand's disease have been affected because of both reduced product exposure and the use of cryoprecipitate, which carries less risk, for statistical reasons, because it is derived from fewer donors.[20] At this writing, the acquired immunodeficiency syndrome had already developed in 7% to 9% of seropositive individuals, and more than half of these persons had already died. This is similar to other cohorts of HIV-infected persons.[15] Human immunodeficiency virus infection has also been unwittingly transmitted to many of the sexual partners of these patients.[21]

NATURE OF THE PROBLEM OF SAFETY FROM VIRAL CONTAMINATION

Plasma-derived coagulation factor concentrates are likely to be contaminated with infectious viruses for several reasons: (1) The products are made from very large pools of plasma, each obtained from up to 8000 donations, with additional pooling of process intermediates. In such large population samples, even rare carriers of infectious diseases are likely to occur. (2) Tests to screen for causal agents in donated plasma may not exist or, when available, are not 100% sensitive. In particular, a specific test for HIV did not exist until 1985, surrogate tests for NANBHV probably detect fewer than 60% of carriers,[22] and HBV can be transmitted despite screening for hepatitis B surface antigen in the nanogram range. In combination with the large number of donations, it becomes likely that plasma pools for fractionation will be contaminated by a small number of donations that either escape detection for pathogens or are released from quarantine at the collection site through clerical error. It is possible that even a single unit of plasma infected with HIV, NANBHV, or HBV contains a sufficient virus titer to render all final container material infectious.[13] (3) Due to the lability of the coagulation factors, the processes used for preparing them are of a gentle nature chemically and are unable to inactivate viruses that happen to be copurified with them during manufacturing. This distinguishes the clotting factor concentrates from albumin and plasma protein fraction, which have been rendered safe by pasteurization for over 30 years. Immunoglobulins prepared by Cohn-Oncley fractionation have also been safe, but the scientific basis seems to be complex, including a combination of virus clearance during fractionation, virus inactivation involving cold ethanol precipitation at some steps in the process, and the presence of antibodies to HBV in the final product.[17]

METHODS OF VIRUS INACTIVATION IN CLOTTING FACTOR CONCENTRATES

The methods of virus inactivation used to prepare commercial clotting factor concentrates are summarized in Table 2.

Heat Inactivation

Although originally developed in an attempt to reduce transmission of hepatitis viruses,[23] heating of lyophilized factor VIII and factor IX has been more effective in reducing transmission of HIV. In general, clotting factors are highly labile to the effects of heating. However, the stability of the proteins (and contaminating viruses) is influenced by the temperature, moisture content, and chemical milieu, including the presence of other proteins or sugars as stabilizers. In general, inactivation of viruses is more rapid in the liquid than the lyophilized state.[24] "Dry-heating" procedures have included exposure to 60°C for 24, 30, 72,[25] and 144 hours, exposure to 68°C for 72 hours, and exposure to 80°C for 72 hours.[26] Only the last three processes are still in use. Modifications of these direct procedures have included suspension of the lyophilizate in *n*-heptane before heating at 60°C for 20 hours[27] and exposure to water vapor at 1190 mbar in nitrogen at 60°C for 10 hours for factor VIII plus 80°C for 1 hour at 1370 mbar for factor IX complex and anti-inhibitor coagulant complex.[28] With the addition of sucrose and glycine as protein stabilizers (analogous to the use of sodium caprylate and *N*-acetyltryptophan to stabilize albumin) it has become possible to "pasteurize" factor VIII in the liquid state at 60°C for 10 hours.[29]

A worldwide survey by the Centers for Disease Control, Atlanta, Ga, of ap-

Table 2.—Methods of Virus Inactivation Used in the Commercial Preparation of Clotting Factor Concentrates

Method of Inactivation	Manufacturer	Product Name(s)	Comment*
"Dry-heated" lyophilizate			
60°C for 24 h	Alpha Therapeutic Corp, Los Angeles, Calif	Profilate HT (dry)	Discontinued
60°C for 30 h	Armour Pharmaceutical Co, Blue Bell, Pa	Factorate HT	Discontinued
60°C for 72 h	Hyland Division, Baxter Healthcare Corp, Glendale, Calif	Hemofil T	Discontinued
60°C for 144 h	Hyland Division, Baxter Healthcare Corp	Proplex T, Autoplex T	Factor IX complex, anti-inhibitor complex
68°C for 72 h	Cutter Biological, Berkeley, Calif	Koate HT, Konyne HT	Factor IX complex
80°C for 72 h	Blood Products Laboratory, Elstree, United Kingdom	NHS, factor VIII (8Y)	Not approved by FDA
		NHS, factor IX (9A)	Not approved by FDA
Heated as a lyophilizate in a modified state			
60°C for 20 h in *n*-heptane	Alpha Therapeutic Corp	Profilate HT (wet), Profilate HP	. . .
		Profilnine HT (wet)	Factor IX complex
60°C for 10 h in vapor	Osterreichisches Institut für Haemoderivate, Vienna, Austria	Kryobulin TIM3	Not approved by FDA
60°C for 20 h and 80°C for 1 h in vapor	Osterreichisches Institut für Haemoderivate	Kryobulin TIM4	Factor IX complex, not approved by FDA
		FEIBA VH	Anti-inhibitor complex
60°C for 72 h in chloroform	Osterreichisches Institut für Haemoderivate	Kryobulin	Discontinued
Heated in solution at 60°C for 10 h with sucrose and glycine	Behringwerke, Marburg, West Germany	Humate P	. . .
	Cutter Biological	Koate HS	. . .
Treated with a solvent-detergent mixture			
Tri(*n*-butyl) phosphate and sodium cholate	New York (NY) Blood Center	Coagulation factor VIIISD	. . .
	American Red Cross, Washington, DC	AHF-SD	. . .
Tri(*n*-butyl)phosphate and Tween 80	Cutter Biological	Koate HP	. . .
	Alpha Therapeutic Corp	Profilate SD	. . .
Treated with beta-propiolactone and ultraviolet light	Biotest, Frankfurt, West Germany	PPSB	Factor IX complex, not approved by FDA
Immunoaffinity purified			
"Dry-heated" at 60°C for 30 h	Armour Pharmaceutical Co	Monoclate	. . .
Treated with tri(*n*-butyl)phosphate and Triton X-100	Hyland Division, Baxter Healthcare Corp	Hemofil M	. . .
	American Red Cross	AHF-M	. . .

*Unless otherwise indicated, the product is a factor VIII concentrate. FDA indicates Food and Drug Administration.

proximately 75 suspected cases of HIV transmission by heat-treated factor concentrates resulted in the identification of 18 cases that met strict criteria for a probable association.[11] The 18 reported cases included 8 seroconversions in patients who received US-manufactured concentrates from screened donations with viral inactivation of the product in the lyophilized state at 60°C for 24 (1 case in the United States) and 30 (7 cases in Canada) hours. Although the Canadian patients also received another product (unscreened, inactivated, "dry heated" at 68°C for 72 hours), careful epidemiologic studies clearly showed a strong association of HIV seroconversion with the receipt of at least one of three lots of the product treated at 60°C for 30 hours; these three lots were all made from a common plasma pool. On the basis of these reports, the manufacturer voluntarily withdrew this product. The product inactivated at 60°C for 24 hours was independently discontinued. No additional product or process could be clearly implicated by analysis of the remaining seroconversion cases involving heat-inactivated products. However, other "dry-heated" products have been voluntarily discontinued because of reported transmission of NANBHV (see below).

Treatment With Chemical Agents

Because of their greater chemical stability compared with factor VIII, it has been possible to develop prothrombin complex concentrate products by nonspecific alkylation with beta-propiolactone in combination with exposure to ultraviolet light.[30] Concerns of the Food and Drug Administration about the safety of these products include the known carcinogenicity of beta-propio-

Table 3.—Estimated Viral Clearance and Results of Clinical Trials for Virally Inactivated Clotting Factor Concentrates*

		Estimated Viral Clearance, $\log_{10}$†			Virus Transmissions in Clinical Trials, No. of Subjects‡		
Method of Inactivation	**Manufacturer**	**HBV**	**NANBHV**	**HIV**	**HBV**	**NANBHV**	**HIV**
"Dry-heated" lyophilizate							
60°C for 30 h	Armour Pharmaceutical Co, Blue Bell, Pa	<3.5	≥3.5	. . .	0/2	2/2	7 proved cases[11]
60°C for 72 h	Hyland Division, Baxter Healthcare Corp, Glendale, Calif	<2.5	. . .	2-4	0/12	11/13	0/13
68°C for 72 h	Cutter Biological, Berkeley, Calif	. . .	≥3.4	≥2.8	. . .	1/6	0/6
80°C for 72 h	Plasma Fractionation Laboratory, United Kingdom	. . .	. . .	. . .	. . .	0/16	0/16
Lyophilizate heated in a modified state							
60°C for 20 h in *n*-heptane	Alpha Therapeutic Corp, Los Angeles, Calif	>2.7	2.7-4	3.2-3.5	0/18	8/37	0/37
60°C for 10 h in vapor	Osterreichisches Institut für Haemoderivate, Vienna, Austria	. . .	. . .	>6.0§	4/14	0/24	0/28
60°C for 72 h in chloroform	Osterreichisches Institut für Haemoderivate	. . .	. . .	≥6.0	0/3	3/3	. . .
Heated in solution at 60°C for 10 h	Behringwerke, Marburg, West Germany	≥4.5	>5.5	≥5.5	0/10‖	0/26	0/26
Treated with a solvent-detergent mixture							
Tri(*n*-butyl)phosphate and sodium cholate	New York (NY) Blood Center	≥4.0	≥4.0	≥4.5	. . .	0/17	0/18
Tri(*n*-butyl)phosphate and Tween 80	New York Blood Center	. . .	≥5.0	≥3.6	. . .	0/13¶	. . .
Treated with beta-propiolactone and ultraviolet light	Biotest, Frankfurt, West Germany	7.0	≥4.5	≥6.0	0/5	0/5	. . .
Immunoaffinity purified							
"Dry-heated" at 60°C for 30 h	Armour Pharmaceutical Co	. . .	. . .	. . .	. . .	0/25	0/25
Treated with tri(*n*-butyl)phosphate and Triton X-100	Hyland Division, Baxter Healthcare Corp	. . .	. . .	. . .	. . .	0/14	0/14

*HBV indicates hepatitis B virus; NANBHV, non-A, non-B hepatitis virus; and HIV, human immunodeficiency virus.

†These data were reported by Horowitz[17] based on a review of the literature by Prince et al.[13]

‡These data were compiled from several sources, including Mannucci and Colombo,[14] Pierce et al,[15] and Horowitz.[17] Each source is a referenced literature review.

§Immuno US has also reported inactivation of HIV of 6.3 $\log_{10}$ or greater in an activated prothrombin complex concentrate, FEIBA, which is treated in vapor at 60°C for 10 hours plus 80°C for 1 hour.

‖Two cases of transmission of HBV by this product have been reported.[36]

¶Gonzaga et al[37] reproted no cases of hepatitis in 13 patients with hemophilia B who received factor IX concentrate that was treated to inactivate viruses.

lactone and the problem of controlling the development of neoantigens, which could sensitize the recipients. More recently, investigators at the New York (NY) Blood Center have developed inactivation procedures using the organic solvent tri(*n*-butyl)phosphate in combination with the detergents sodium cholate, Tween 80 ,and Triton X-100.[31,32] This procedure removes the lipid envelopes of viruses that contain such a coat. Studies in chimpanzees carried out before the product was used in man[31] confirmed a high level of inactivation of HIV, HBV, and NANBHV.

Immunoaffinity Purification

Factor VIII has also been purified by passage through an immunoaffinity column containing a murine monoclonal antibody.[33] Material purified in this way can achieve near homogeneity, with a clotting factor activity as high as 4500 IU per milligram of protein (before dilution with albumin), compared with an average activity of 1 IU per milligram of protein for other concentrates. A corresponding reduction in the virus burden of 3 to 4 $\log_{10}$ infectious units of various agents occurs. Because viral clearance by the column process is incomplete, manufacturers additionally treat the product by either heating at 60°C for 30 hours in the lyophilized state or prior treatment with a solvent-detergent mixture.

Recipients of concentrates show immunologic abnormalities, particularly elevations of the $CD8^+$ T lymphocyte subset, which is thought to be indicative of immune activation and/or chronic suppression.[15] It is not known to what extent these abnormalities are due to undiagnosed viral diseases. It has been proposed that highly purified clotting factor concentrates are less likely to cause chronic immunostimulation. This might be beneficial, especially to patients with HIV infection, because of the hypothesized association of HIV expression in vivo with immune stimulation and acute infection.[34] Although some studies suggest resolution of anergy and a decreased rate of decline in the number of $CD4^+$ T lymphocytes HIV-infected hemophiliacs undergoing exclusive therapy with affinity-purified factor concentrate,[35] these results are preliminary and have not been confirmed by other workers. Some scientists believe that purified clotting factors should be preferred on general principle because they are less likely to transmit undiscovered infectious agents and less likely to produce as yet undefined toxicities.

MEASURING THE SAFETY OF CURRENT CLOTTING FACTOR CONCENTRATES

The available data on viral inactivation and clinical safety of inactivated clotting factor concentrates are summarized in Table 3, based on recent reviews of the literature.[14,15,17]

Among HIV, HBV, NANBHV, and delta agent, only HIV infectivity can be studied in tissue culture. Therefore, the ability of current manufacturing processes to eliminate HBV and NANBHV infectivity from clotting factor concentrates has been assessed in chimpanzees by spiking in-process materials with known titers of these agents and then challenging the animals with the resultant products. Human immunodeficiency virus inactivation has been studied in tissue culture systems by the use of similar spiking experiments. No quantitative studies of the inactivation of hepatitis delta agent have been carried out. Generally, these assessments are limited by the achievable concentration of the virus inoculum and by the sensitivity of the assay method. Also, it is not known how to relate the infectious dose in chimpanzees or in tissue cultures to that in man. Nevertheless, these data serve to establish lower boundaries for the effectiveness of viral inactivation by the process in question. Comparisons between processes must be made with caution since there are numerous methodologic differences that affect the results. Overall, reports suggest that significant viral killing is achieved by beta-propiolactone in combination with ultraviolet light, pasteurization, solvent-detergent, wet vapor heating, and dry-heating or modified dry-heating procedures. Monoclonal affinity purification in combination with other inactivation procedures also has demonstrated a high degree of viral clearance. It is noteworthy that despite differences in manufacturing, the in vivo recovery of factor VIII is comparable for products virally inactivated by these methods.[12,33]

Clinical experience is the ultimate test of product safety. The summarized studies suffer from numerous limitations: (1) small size due to the limited number of subjects free of hepatitis and HIV; (2) lack of standardization of the study populations, such as inconsistent exclusion of patients with previous product exposures or variations in use of HBV vaccination; (3) inconsistency in the criteria for detection and diagnosis of non-A, non-B hepatitis; and (4) inadequate assessment of lot-to-lot variations. Their small size alone implies that they cannot prove safety to a high degree of significance. Concern over the lack of a standard and scientifically rational approach to clinical evaluation of hepatitis from clotting factor concentrates led to a recommendation for uniform criteria by the International Committee on Thrombosis and Hemostasis in 1984.[38] With the benefit of these criteria and despite the many other limitations, estimates for rates of transmission of NANBHV are useful in distinguishing among the current products and clearly establish an improvement over previous concentrates. In particular, transmission of NANBHV has been shown for products heated in the lyophilized state under all except the most severe conditions or with suspension in heptane.[39-43] By contrast, pasteurization, hot vapor treatment, extreme dry heating, solvent-detergent treatment, and affinity purification in combination with other procedures seem to have reduced the infectivity of NANBHV.[15,17,44-46] While the absence of transmission of HIV in such small trials is of limited predictive value, it does confirm a dramatic improvement since 1984. International surveillance efforts by the Centers for Disease Control of nearly 1500 patients who were seronegative in November 1985 suggested that the risk of HIV infection since 1985 may be less than 1 per 1000 patient-years of therapy.[11] Safety for HBV is also difficult to evaluate from the reported studies, but, again, transmission is clearly reduced compared with the previous generation of products. Vaccination against HBV remains appropriate to assure protection.

ALTERNATIVES TO PLASMA-DERIVED CLOTTING FACTORS

Several alternatives exist to the use of clotting factor concentrates derived from human plasma. For many patients with mild or moderate hemophilia or von Willebrand's disease, some bleeding episodes can be controlled by the infusion of desmopressin, a synthetic analogue of vasopression that produces transient elevations of factor VIII and von Willebrand factor.[47] Also, a porcine factor VIII (Porton Products, Los Angeles, Calif) is commercially available, but only for treating patients with inhibitors. The goal of a safe and pure recombinant clotting factor was brought closer by the recent report of successful therapy without toxicity for two patients with classic hemophilia who were given a product derived from cultures of Chinese hamster ovary cells transfected with DNA containing the coding region for human factor VIII.[48] Preliminary results of expanded clinical trials support the impression that recombinant factor VIII is both safe and effective.[49,50]

CONCLUSION

The introduction of virus-inactivating procedures into manufacturing has remarkably improved the safety of commercially prepared clotting factor concentrates since 1985. Human immunodeficiency virus transmission has virtually ceased, and some processes now exist that appear capable of inactivating NANBHV and HBV as well. Only long-term surveillance of large numbers of persons who have exclusively received products treated to inactivate viruses will reveal the level of infectious risk of these products. However, the available data suggest that few if any HIV infections are likely to occur. Residual risk of hepatitis B should still be controlled with vaccination, if only because patients with bleeding disorders remain at risk from other blood products. Products made by processes that do not inactivate NANBHV can and should be replaced in the marketplace, even though it is possible that transmission of NANBHV may be reduced by donor screening for a specific agent in the near future. The additional medical value of products with a high degree of protein purity remains to be established.

These amazing strides have been accomplished at a price. The end user cost of clotting factor replacement has risen an average of sixfold in 2 years, from approximately $10000 to $60000 per year.[15] This is due to the combined effects of a reduced supply, resulting from manufacturing products at less yield due to the addition of procedures for inactivation of viruses, and the added manufacturing costs of the new methods. Also, costs have been driven by a reduced demand for albumin, causing factor VIII to become the main product supporting plasma fractionation. Because of these dramatically increased costs, patients have been less able to afford both factor therapy and comprehensive care. In addition, a serious shortage of clotting factor concentrates was experienced in 1988, when many manufacturers shifted production away from dry-heated products. Hopefully, competition in the marketplace along with cooperative efforts by government, industry, health care providers, and third party payers will overcome the crises of supply and cost so that the full benefits of safer clotting factor concentrates will be shared by all patients.

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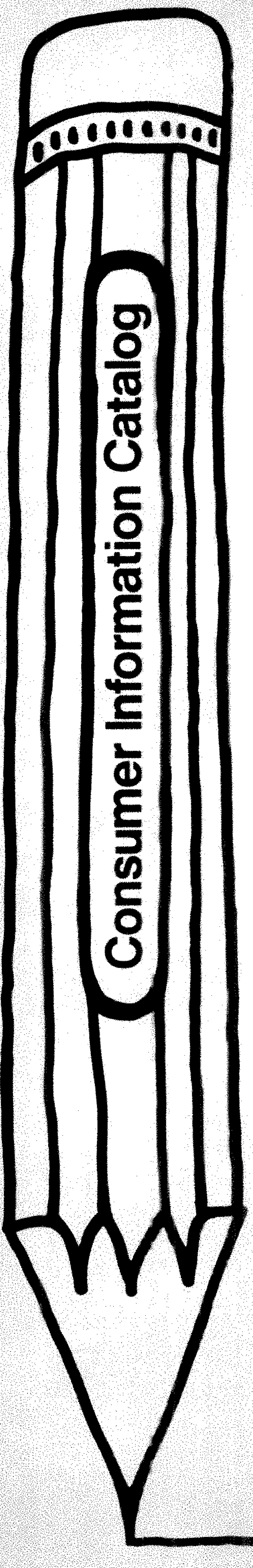

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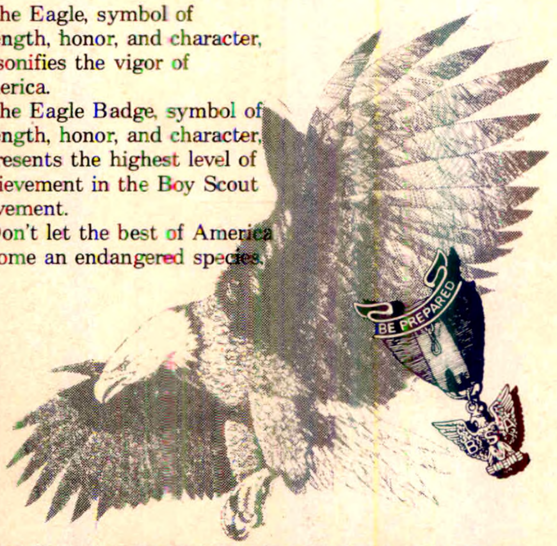

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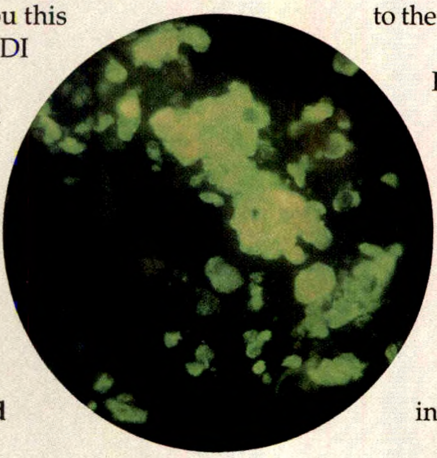

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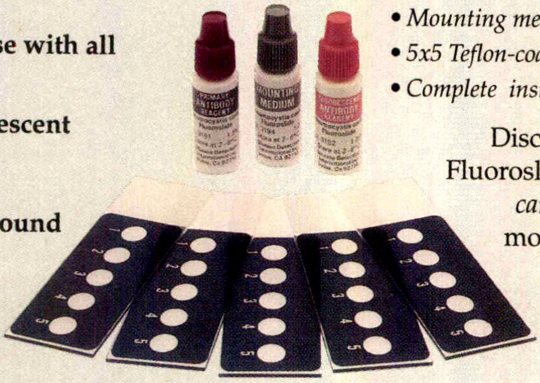

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HIV antigen typically disappears following seroconversion but reappears in the latter stages of the disease. The reappearance of antigen usually indicates impending clinical deterioration. An asymptomatic HIV-antigen-positive person is six times more likely to develop AIDS within three years than a similar individual who is HIV antigen negative.[3] Thus, testing for the presence of HIV antigen assists physicians in monitoring the progression of the disease.

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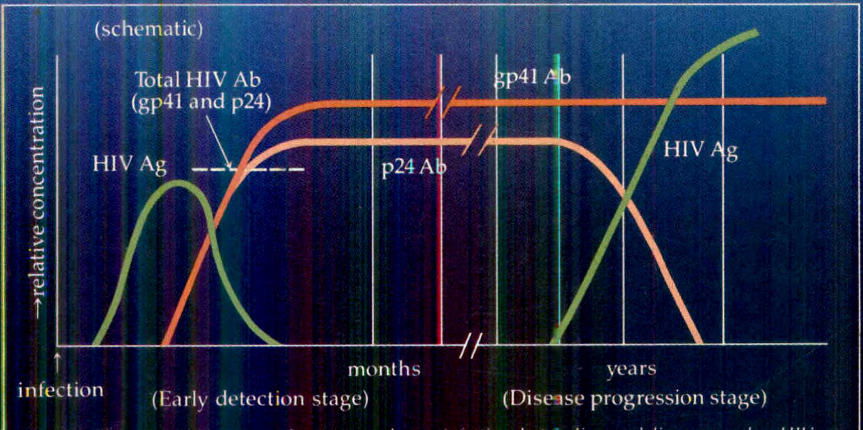

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LETTERS TO THE EDITOR

638 **Biclonal Composite Lymphoma**
Laura E. Sulak, MD; Fiona E. Craig, MD;
Milka M. Montiel, MD;
Peter M. Banks, MD, San Antonio, Tex
In Reply
Ricardo E. Duque, MD, Birmingham, Ala;
Eric T. Everett, MT(ASCP), Charleston, SC;
Jose Iturraspe, MT(ASMT), Gainesville, Fla

Revisiting the Autopsy
Syed A. Hoda, MD, New Orleans, La

An Erroneous Identification of the Pathergic Granuloma
Robert Fienberg, MD, Boston, Mass
In Reply
Masayuki Shintaku, MD,
Susumu Ikehara, MD, Osaka, Japan

In Situ Hybridization for Detection of Cytomegalovirus
John G. Strickler, MD; Cedith M. Copenhaver, MS;
Dale C. Snover, MD, Minneapolis, Minn

EDITORIAL

641 **Adenomatoid Tumors: Adrenal Cysts and Being First**
Gene P. Siegal, MD, PhD, Chapel Hill, NC

SPECIAL ARTICLE

643 **Neuropathology of the Acquired Immunodeficiency Syndrome**
Umberto De Girolami, MD, Thomas W. Smith, MD, Worcester, Mass;
Dominique Hénin, MD, Jean-Jacques Hauw, MD, Paris, France

ORIGINAL ARTICLES

656 **Vascular Transformation of Lymph Node Sinuses: A Process Displaying a Spectrum of Histologic Features**
Mary L. Ostrowski, MD; Tariq Siddiqui, MD; Robert E. Barnes, MD;
Marcia J. Howton, MD, Jacksonville, Fla

661 **Ultrastructural Morphometry in the Diagnosis of Sézary Syndrome**
Claire M. Payne, PhD, Lewis Glasser, MD, Tucson, Ariz

672 **Papillary Cystadenoma of the Epididymis: A Report of Three Cases With Lectin Histochemistry**
Peter J. Kragel, MD, Bethesda, Md;
Joseph Pestaner, Baltimore, Md;
William D. Travis, MD, W. Marston Linehan, MD, Bethesda, Md;
Michele R. Filling-Katz, MD, Louisville, Ky

Continued on page 634

Continued from page 633

676 Lymphoblastic Crisis of Chronic Myelogenous Leukemia: Hand Mirror Variant
Areta Kowal-Vern, MD; Bailey A. Birdsong, MD;
George Dizikes, PhD; Ruta Radvany, PhD;
Surbhi N. Desai, PhD; Harold Schumacher, MD, Maywood, Ill

679 Mixed Gonadal Dysgenesis: A Review of 15 Patients Reporting Single Cases of Malignant Intratubular Germ Cell Neoplasia of the Testis, Endometrial Adenocarcinoma, and a Complex Vascular Anomaly
Thomas M. Wallace, MD,
Howard S. Levin, MD, Cleveland, Ohio

689 Morphometric Analysis of Germinal Centers in Nonthymomatous Patients With Myasthenia Gravis
Cesar A. Moran, MD, New York, NY;
Saul Suster, MD, Miami Beach, Fla;
Joan Gil, MD; Jaishree Jagirdar, MD, New York, NY

692 Granular Cell Tumor: Immunohistochemical Analysis of 21 Benign Tumors and One Malignant Tumor
Michael T. Mazur, MD; Jana J. Shultz, MS;
Jeffrey L. Myers, MD, Birmingham, Ala

697 Palisading Granulomas Associated With Lung Cancer
Arnost Kolin, MD, FRCPC,
Tadaaki Hiruki, MD, Toronto, Ontario

700 Carcinoid Tumors of the Duodenum: A Clinicopathologic Study of 99 Cases
MAJ Allen P. Burke, MC, USAF; Leslie H. Sobin, MD;
Birgitte H. Federspiel, MD; LTC Kris M. Shekitka, MC, USAF;
Elson B. Helwig, MD, Washington, DC

705 Fine-Needle Aspiration Cytology of Malignant Hemangiopericytomas With Ultrastructural and Flow Cytometric Analyses
Kim R. Geisinger, MD, Winston-Salem, NC;
Jan F. Silverman, MD, Greenville, NC;
James O. Cappellari, MD, Winston-Salem, NC;
David J. Dabbs, MD, Greenville, NC

711 Detection of Epstein-Barr Virus Genomes in Archival Tissues by Polymerase Chain Reaction
Stephen C. Peiper, MD; Jeffrey L. Myers, MD;
Elaine E. Broussard, MT(ASCP), Birmingham, Ala;
John W. Sixbey, MD, Memphis, Tenn

715 Heterogeneity of Common Hematologic Parameters Among Racial, Ethnic, and Gender Subgroups
Sunita Saxena, MD, Edward T. Wong, MD, Los Angeles, Calif

TEACHING PATHOLOGY AND LABORATORY MEDICINE

720 On the Fifth Year, Subspecialty Certification, and Delayed Gratification: A Partial Solution
John C. Neff, MD, Columbus, Ohio

Continued on page 636

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Continued from page 634

BRIEF REPORTS

722 **Adenomatoid Tumor of the Adrenal Gland With Ultrastructural and Immunohistochemical Demonstration of a Mesothelial Origin**
William D. Travis, MD, Bethesda, Md;
Ernest E. Lack, MD, Norio Azumi, MD, Washington, DC;
Maria Tsokos, MD, Jeffrey Norton, MD, Bethesda, Md

725 **Adenomatoid Tumor of the Adrenal Gland**
Preston R. Simpson, MD, Fresno, Calif

727 **Fluoxetine and the Bleeding Time**
John E. Humphries, MD; Munsey S. Wheby, MD;
Scott R. VandenBerg, MD, PhD, Charlottesville, Va

728 **Small-cell Carcinoma of the Vagina: A Clinicopathologic Study**
James J. Rusthoven, MD, FRCPC,
Dean Daya, MD, FRCPC, Hamilton, Ontario

731 **Adenocarcinoma of the Uterine Cervix of Small Intestinal Type Containing Numerous Paneth Cells**
Kenneth R. Lee, MD, Thomas D. Trainer, MD, Burlington, Vt

ADVANCES IN SCIENCE AND PATHOLOGY

734 **Image Cytometry**
Dennis W. Ross, MD, PhD, Chapel Hill, NC

REGULAR DEPARTMENTS

734 **News and Comment**

735 **Books**
Principles and Practice of Infectious Diseases (Mandell, Douglas, Bennet, eds)
Reviewed by John A. Bryan, MD, Atlanta, Ga
Progress in AIDS Pathology (Rotterdam, Sommers, Rácz, Meyer, eds)
Reviewed by Azorides Rafael Morales, MD, Miami, Fla

736 **Classified Advertising**

637 **Instructions for Authors**

CORRECTIONS

642 **Acute Congestive Heart Failure in a 55-Year-Old Man: Rheumatic Carditis Diagnosed by Endomyocardial Biopsy**
Peggy L. Byck, MD; Catherine M. Listinsky, MD;
Terry B. Cooper, MD;
Silvio E. Papapietro, MD, Birmingham, Ala

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Biclonal Composite Lymphoma

To the Editor.—We read with interest the article by Duque, Everett, and Iturraspe[1] on the flow cytometric analysis of a biclonal composite lymphoma. Recently, we were able to exhaustively study a supraclavicular lymph node from an 85-year-old man, with results that support the observations of these authors, using immunologic, histologic, and genetic techniques. We detected two distinct cell populations by flow cytometry in a cell suspension of the lymph node using simultaneous forward-angle light scatter and side scatter. Utilizing selective gating techniques, we defined a population of large cells with expression of surface membrane immunoglobulin M-kappa (Sm IgM-kappa) while the majority of the cells (approximately 80%) were small and expressed Sm IgG-lambda. Immunostaining of tissue frozen sections confirmed these results, directly correlating larger and smaller cell types morphologically with the immunoglobulin profiles detected by flow cytometry.

The diagnosis of composite lymphoma as defined by Custer[2] and modified by Rappaport et al[3] and Kim et al[4] has traditionally required a clearly demonstrable delineation between two morphologically different lymphomas. However, unlike previously reported cases of immunologically proven biclonal B-cell composite lymphomas,[5-7] histologic examination of the lymph node in our case revealed a complex intermingling of the two distinct cellular populations—one characteristic of small lymphocytic lymphoma and the other of large-cell lymphoma. DNA analysis of the neoplasm was performed by Southern blot method using a probe to the joining region of immunoglobulin heavy chain gene (JH). Three discrete nongermline bands were identified, and although not independently conclusive, this pattern was considered to be compatible with the biclonal nature of this lymphoma.

We agree with the conclusion of the authors that the use of immunophenotypic, genotypic, and cell cycle characteristics allow for more objective characterization of complex processes such as these. Furthermore, we suggest that the concept of composite lymphomas should be expanded to include neoplasms such as the one we describe in which the cellular populations are histologially intermixed, but can be shown to be of separate clonal origin by immunologic or genetic techniques.

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1. Duque RE, Everett ET, Iturraspe J. Biclonal composite lymphoma: a multiparameter flow cytometric analysis. *Arch Pathol Lab Med.* 1990; 114:176-179.

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In Reply.—We agree with Dr Sulak and her colleagues that complex lymphomatous processes are not always amenable to morphologic assessment. We would like to add that in the case we described[1] there was also complex intermingling of the two processes in addition to areas of "collision." We recommend performing not only immunophenotypic analysis in lymphoma but DNA ploidy and cell cycle analysis on a routine basis. These techniques can be performed simultaneously and provide additional information.

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1. Duque RE, Everett ET, Iturraspe J. Biclonal composite lymphoma: a multiparameter flow cytometric analysis. *Arch Pathol Lab Med.* 1990; 114:176-179.

Revisiting the Autopsy

To the Editor.—The editorial by Henson[1] is a timely one. The case made for revisiting the autopsy is a compelling one, and the necessity of emphasizing and reinstating the "scientific approach" is presented with refreshing wisdom. There is no doubt that autopsies will continue to play a critical role in the practice of medicine; however, it needs to be tailored to the needs of modern medicine if we are to retain the clinician's interest.

We should strive to make autopsies more "problem-oriented." Perhaps, more than anything else, our ability to expeditiously find solutions to unresolved clinical puzzles would earn the appreciation of our clinical colleagues. Performing "limited" autopsies may be another way to generate interest in autopsies, eg, the autopsy may be limited to the brain in a case of an astrocytoma. This approach may also kindle a greater willingness among the bereaved to authorize autopsies. Another approach, in selected cases, would be to get postmortem needle biopsy specimens of various organs. Even postmortem fine-needle aspiration may be tried in certain instances. These "alternative" approaches to autopsies may be popular among pathologists who no longer want to perform autopsies, and may be ideas for which the time has come. Perhaps "problem-oriented" autopsies would make revisiting the morgue a much more frequent and worthwhile exercise for clinicians and pathologists alike.

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1. Henson DE. Revisiting the autopsy. *Arch Pathol Lab Med.* 1990;114:127-128.

An Erroneous Identification of the Pathergic Granuloma

To the Editor.—Shintaku et al[1] in the November 1989 issue of the Archives have described sarcoidlike granulomas with systemic angiitis. A careful scrutiny of Fig 1 discloses a pathergic granuloma[2,3] with central pathergic necrosis and an eccentric palisading characteristic of pathergic (Wegener's) granulomatosis.[4] The diffuse granulomatous tissue, with scattered giant cells in Fig 2, is also characteristic of the same disorder. The involvement of lymph nodes, while not frequent in this type of granulomatosis, has been described previously.[5] The pulmonary hemorrhage was due to a capillaritis, a feature that again, while not frequent, is characteristic of pathergic (Wegener's) granulomatosis, particularly when associated with pathergic granulomas.[6] The crescentic glomerulonephritis further supports this diagnosis. Clinically, the case fits nicely into the category of pathergic granulomatosis. No biopsy specimen was available, but in the light of the interpretation of Shintaku et al, even with a biopsy specimen the failure to distinguish between a palisading necrotizing pathergic granuloma and the compact circumscribed nonpalisading sarcoid granuloma would have precluded the correct treatment with a cytotoxic drug such as cyclophosphamide, which might have been life saving. At present, of course, the diagnosis would have been facilitated by the use of the antineutrophil cytoplasmic antigen (ANCA) test.

Robert Fienberg, MD
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In Reply.—We thank Dr Fienberg for his comments regarding our recent article. We, however, cannot agree with his opinion. The basic pathologic feature of our case is "epithelioid cell" granulomatosis, which involves predominantly intrathoracic lymph nodes, lungs, aorta, and kidneys. Granulomatous inflammatory findings with fibrinoid necrosis such as Wegener's granulomatosis were not seen in systemic blood vessels. In addition, palisading arrangements of histiocytes surrounding necrotic foci were not observed in our case; epithelioid cells shown in Fig 1 of our article apparently do not form characteristic palisading borders.

As discussed in our article, we, of course, considered the diagnostic possibility of Wegener's granulomatosis in this case. However, the remarkable hilar lymphadenopathy due to typical epithelioid cell granulomas, the absence of necrotizing or ulcerating lesions in the respiratory tract, and the lack of fibrinoid necrosis in the arterial lesions are hardly consistent with findings of pathergic (Wegener's) granulomatosis.

We consider that allergic diathesis or hypersensitivity reaction has probably played an important role in rapid progression of the patient's clinical course, and we think that some exudative changes observed in the wall of small- to medium-sized vessels are a reflection of this condition. However, clinicopathologic features of this patient are, as a whole, quite different from those of Wegener's granulomatosis.

The pulmonary hemorrhage might have been caused by "pulmonary capillaritis," as Fienberg suggested, but we do not think that this justifies the diagnosis of Wegener's granulomatosis.

After this article was submitted to the Archives, an article dealing with a similar case appeared in another journal, in which the clinicopathologic features were highly similar to our case,[1] though not associated with glomerulonephritis and pulmonary hemorrhage. The etiopathogenesis of such an extremely rare combination of sarcoidlike granuloma and systemic angiitis will be elucidated with the accumulation of detailed clinicopathologic reports of similar cases.

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In Situ Hybridization for Detection of Cytomegalovirus

To the Editor.—We read with interest the report by Clayton and colleagues,[1] which correlates in situ hybridization (ISH) with histology and viral culture for detecting cytomegalovirus (CMV) in endoscopic colonic biopsy specimens. The authors conclude that ISH, which usually confirms the diagnosis of CMV colitis when inclusions are identified, only rarely demonstrates staining when inclusions are not suspected by histologic examination. Our ISH studies of various autopsy tissues have yielded similar results.[2] We now briefly describe our experience using ISH to detect cytomegalovirus in open lung biopsy specimens from 21 bone marrow transplant patients with clinical and culture evidence of CMV infection.

In 15 lung biopsy specimens, histologic examination revealed characteristic CMV inclusions, and culture of 14 of the 15 biopsy specimens confirmed the diagnosis of CMV pneumonitis. In situ hybridization, performed with a biotinylated CMV probe (Enzo Biochem), detected infected cells in 12 of these 15 biopsy specimens; the other 3 biopsy specimens showed no viral inclusions in the actual sections studied by ISH. We conclude that ISH usually detects CMV-infected cells in lung biopsy specimens with identifiable CMV inclusions, but sampling error, even in wedge biopsy specimens, may cause false-negative ISH results. These conclusions are in agreement with previous studies of CMV infections of the colon[1] and other organs.[2]

In 6 of the 21 lung biopsy specimens, examination of multiple sections showed no CMV inclusions. Three of these biopsy specimens had positive viral cultures, and ISH detected rare CMV-infected cells in two of these cases. The other three biopsy specimens without viral inclusions had negative cultures and ISH results, although cultures from other sites indicated that these patients were infected with CMV. We conclude that in situ hybridization may occasionally detect CMV-infected cells when viral inclusions are not identified. We therefore agree with the suggestion of Clayton and colleagues[1] that in situ hybridization may be justified in tissues from patients who are strongly suspected of having a CMV infection, even if routine histologic studies are negative.

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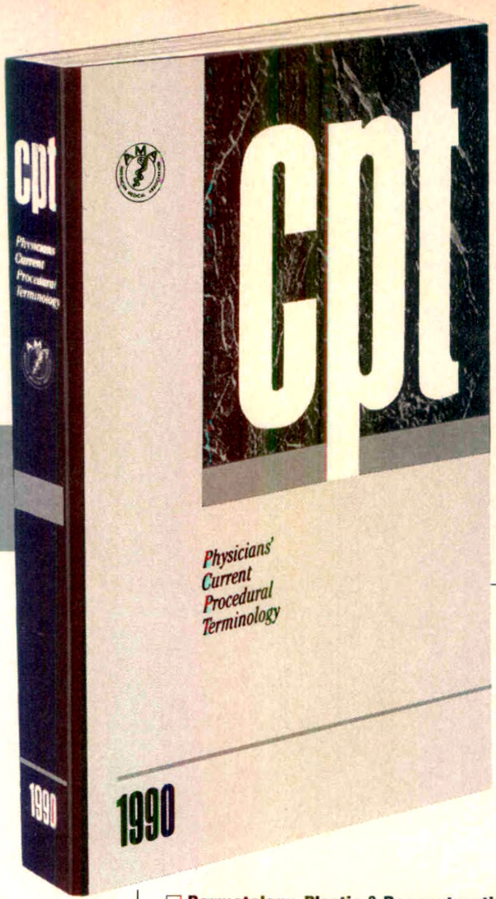

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Editorial

Adenomatoid Tumors

Adrenal Cysts and Being First

Gene P. Siegal, MD, PhD

Daughter 1: "I'm first."
Daughter 2: "Why?"
Daughter 1: " 'Cause I called it first!"

. . . and so begins another day at our home and probably most other homes with children. Being first controls everything from reading the Sunday comics to preferential receipt of the parental good night "kiss and tuck into bed." Being first means something—it's better than being second—it's competition, it's America!

See also pp 722 and 725.

Being first certainly controls our lives as scientists. Who has not received the admonition, from those in the know, that grants, prizes, and awards in science are not given for being a runner-up. Readers of "cutting edge" journals such as *Nature* or *Science* cannot help but notice a trend where individuals seemingly geographically separated simultaneously publish what appear to be obscure findings; ie, "Genomic DNA from Albanian green-striped vole viruses codes for oncogene sequences with homology to street addresses in Deadwood, South Dakota." This happens for at least two major reasons: knowledge does not follow the laws of Brownian Motion and individuals who work in narrow fields of study communicate with each other. It follows that these individuals may reach agreement as to when and where to submit original work concurrently, thereby obtaining dual priorities for discoveries (and their subsequent rewards). When working well, this technique is invisible to the casual observer but when that twinge of one-upsmanship comes into play, cold fusion in Utah or human immunodeficiency virus isolation in Bethesda (Md) and Paris (France) roars to the forefront.

It therefore caught the collective eye(s) of the editors when two articles arrived at our editorial offices, on the same day, reporting the "first" observation of a relatively common neoplasm (adenomatoid tumor) in an exceedingly uncommon site (the adrenal gland).[1,2] As editors we took the somewhat unusual step of having both articles reviewed together. After appropriate revision they were accepted for publication and are found in this issue of the ARCHIVES (see pages 722 and 725). Subsequent follow-up revealed the authors were unaware of each others' work and so their simultaneous publication appears to be a happy coincidence.

Adenomatoid tumors themselves have enjoyed a long history. Credit for the first description of the lesion as a pathological entity is given to Golden and Ash.[3] A not very rigorous review of the literature will, however, show that adenomatoid tumor was well described (in French) and illustrated several years earlier by Mason and associates.[4] More recently, attempts to describe the "true histogenesis" of adenomatoid tumors have been made by techniques ranging from light microscopy, through histochemistry and immunohistochemistry, to scanning electron microscopy.[5-7] These lesions are common enough today to be diagnosable by fine-needle aspiration cytology[8] and variants have begun to be described.[9]

Similarly, adrenal cysts and pseudocysts (hemorrhagic cysts) have enjoyed a number of firsts over several centuries. Contemporary articles credit Greiselius in 1670, as providing the first description.[10] "Modern" classification schemes of adrenal cysts have been around for decades, although individuals continue to review and rediscover them.[11] Adenomatoid tumors are usually solid with varying amounts of cystic formation. It would seem, therefore, reasonable to include adenomatoid tumors in the differential diagnosis of cystic lesions of the adrenal gland.

A number of years ago we showed, using immunohistochemical techniques, that some pseudocysts are vascular in origin[12] (although we wisely did not claim to be first). Others have subsequently confirmed and expanded these

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observations.[13,14] It would not be unexpected that immunostaining adenomatoid tumors with antibodies directed against components of the basement membrane might give overlapping findings with vascular-derived tumors. Caution should therefore be observed and an appropriate antibody panel employed when both entities are in the differential diagnosis.

To come full circle (without for a moment trying to negate or destroy that exhilaration one feels when making a truly novel observation), claiming to be "first" for clinicopathologic discoveries appears to be a bad practice. Experience has taught us that the fastest way to generate letters to the editor is to claim priority. Hostile letters claiming "we reported this finding 27 years ago in a journal published in Serbo-Croatian or in the *New Zealand Journal of Dance and Histopathology*" are entertaining but not really of much value. The authors[1,2] themselves in these articles rightfully point out that adenomatoid tumors of the adrenal gland have been previously described as such or under alternative terms (eg, see reference 15). Before would-be letter writers apply pen to paper let me be the first to acknowledge that this is not the first time an individual has written about this topic, although others have had a somewhat more cynical and bilious tongue.[16] Enough said!

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CORRECTION

In the article entitled "Acute Congestive Heart Failure in a 55-Year-Old Man" published in the May 1990 issue of the ARCHIVES (1990;114:526-527), a subtitle should have been added to read as follows: "Rheumatic Carditis Diagnosed by Endomyocardial Biopsy."

Special Article

Neuropathology of the Acquired Immunodeficiency Syndrome

Umberto De Girolami, MD; Thomas W. Smith, MD;
Dominique Hénin, MD; Jean-Jacques Hauw, MD

• This review attempts to assess critically the literature on the neuropathology of acquired immunodeficiency syndrome in light of our experience with 172 patients with acquired immunodeficiency syndrome who underwent extensive postmortem examinations of the central and peripheral nervous systems. The neuropathologic manifestations of the disease can be divided into three categories: (1) primary or putative/indirect effects of the human immunodeficiency virus, (2) opportunistic infections, and (3) neoplasms. We discuss the known etiologic agents and postulated pathogenetic mechanisms responsible for the broad range of neurologic diseases observed in patients with acquired immunodeficiency syndrome.

(*Arch Pathol Lab Med.* 1990;114:643-655)

The acquired immunodeficiency syndrome (AIDS) is a systemic viral disease caused by the human immunodeficiency virus (HIV)—a 100-nm RNA virus of the retrovirus group.[1,2] It has been estimated that up to 50% of patients with AIDS experience neurologic dysfunction during the course of the illness, and postmortem evidence of nervous system disease may be discovered in as many as 80%.[3,4] In some patients, neurologic manifestations predominate.[5]

Neuropathologic abnormalities in patients with AIDS can be divided into three broad groups, with considerable overlap between them in given cases: (1) HIV encephalitis and related diseases, (2) opportunistic infections, and (3) neoplasia.[6-20] Furthermore, several distinctive pathologic entities have been described in patients with AIDS who have clinical manifestations referable to the spinal cord,[21-23] peripheral nerve, or skeletal muscle.[24-28] The range of histopathologic abnormalities of the central and peripheral nervous systems of children with the disease varies from what is observed in adults.[29-32] Lastly, it is now becoming clear that different etiologic agents and pathogenetic mechanisms underlie the different clinical and pathologic pictures.[33,34]

This review is based on the study of cases reported in the literature and on our own detailed neuropathologic autopsy study of 172 cases at the New England Deaconess Hospital, Boston, Mass, the University of Massachusetts, Worcester, and the Hôpital de La Salpêtrière, Paris, France. The neuropathologic aspects of AIDS will be discussed in turn by using the following classification:

- Primary or putative/indirect effect of HIV
  - HIV encephalitis[3,4,19,35-38]
  - HIV leukoencephalopathy[39-45]
  - Vacuolar myelopathy[21,22]
  - Neuropathy[24,25,27,28,46-49]
  - Myopathy[24,26,28,50]
  - Vascular disease[51,52]
  - AIDS-dementia complex[34,43,44]
  - Pediatric disease[31,32]
- Opportunistic infections
  - Parasites: toxoplasmosis[53-57]
  - Fungi, mycobacteria, and spirochetes
    - *Aspergillus*, *Candida*, and *Cryptococcus*[58]
    - *Mycobacterium avium-intracellulare*[16,19]
    - *Treponema pallidum*[59]
  - Viral infection
    - Cytomegalovirus (CMV)[60-68]
    - Herpes simplex[69,70]
    - Herpes zoster[71-73]
    - Polyomavirus (progressive multifocal leukoencephalopathy [PML])[74-76]
- Neoplasms
  - Lymphoma[6,77-81]
  - Kaposi's sarcoma[82]

Accepted for publication April 24, 1990.

From the Departments of Pathology and Neurology, University of Massachusetts Medical Center, Worcester (Drs De Girolami and Smith), the Department of Pathology, New England Deaconess Hospital and Harvard Medical School, Boston, Mass (Dr De Girolami), and the Laboratoire de Neuropathologie Raymond Escourolle, Hôpital de La Salpêtrière and University of Paris (France) (Drs De Girolami, Hénin, and Hauw). Dr De Girolami is on sabbatical leave from the University of Paris.

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PRIMARY OR PUTATIVE EFFECT OF HIV ON THE NERVOUS SYSTEM

HIV Encephalitis

Macroscopic examination of the brain is usually not remarkable. The meninges are clear. There may be some ventricular dilatation and widening of the sylvian fissures and adjacent cortical sulci. Microscopically, the inflammatory reaction is characterized by diffuse infiltration of the brain with microglial nodules. Microglial nodules consist of aggregates of microglial cells/macrophages associated with foci of tissue necrosis and pallor of myelin staining (Fig 1). The nodules are observed in the vicinity of small blood vessels and occur concomitant with intraparenchymal pigment-laden macrophages. In the affected areas, the nuclei of capillary and venular endothelial cells may be numerous, large, and misshappen. They are not associated with extensive necrosis of brain parenchyma, and they are ordinarily not accompanied by lymphocytic or plasmacellular infiltrates, either perivascularly or within the parenchyma. The degree of reactive astrocytosis varies with the severity and duration of the lesion and can sometimes be extensive.

Microglial nodules are found diffusely throughout the cerebrum, diencephalon, brain stem, cerebellum, and spinal cord in decreasing incidence.[19] In our experience, they are more frequent in the white matter than the gray matter. An important though not invariable component of the microglial nodule is the multinucleated cell. These are macrophage-derived giant cells that measure 15 to 25 μm in diameter as they occur in the in situ and tissue culture cytopathic reaction to certain viral infections (eg, measles). They differ from foreign body or Langhans-type giant cells because of their smaller size, scant cytoplasm, and the haphazard arrangement of the nuclei.[42,83,84] They can occur admixed with other cells in the microglial nodule or in isolated clusters. In an exceptional case, we have observed them within the subarachnoid space associated with a pronounced fibroblastic response (Fig 2). Some have considered their presence to be a hallmark of HIV infection of the brain[11,42,85]; others find them infrequently.[19]

Meningeal involvement is variable and correlates with either the stage or the severity of the disease. There have been relatively few reports that describe the neuropathologic changes in the early stages of HIV infection of the central nervous system (CNS). An acute encephalopathy has been described clinically coincident with seroconversion,[86] and HIV can be recovered from the cerebrospinal fluid of seropositive individuals with or without neurologic symptoms.[87] Postmortem examination of an asymptomatic HIV seropositive man who died suddenly in a motor vehicle accident showed a mild chronic meningitis without immunocytochemical evidence of HIV or CMV in the brain.[88] The case reported by McArthur et al[89] of a patient with early-onset HIV-related cognitive dysfunction who died suddenly 4 months after the onset of symptoms showed, at postmortem examination, only slight pallor and astrocytosis of the cerebral white matter but no evidence of meningeal inflammation. In severe encephalitis occurring in the latter stages of the disease, giant cells and chronic meningitis may be present.

Human immunodeficiency virus has been isolated from the cerebrospinal fluid and the brain tissue of patients with AIDS.[87,90-92] The identification of the type of cell infected by HIV within the nervous system has been sought by utilizing techniques of viral cocultivation,[93] immunohistochemistry,[30,42,94-98] in situ hybridization,[42,92,97,99] and transmission electron microscopy.[42,100-102] The localization of HIV to the mononucleate and multinucleate macrophages associated with microglial nodules in the brain now appears to be well established.[93,95-98] Furthermore, HIV has been shown to have a selective tropism to the CD4 protein of helper T cells that it uses as a binding site.[103] Staining with antisera against herpes simplex virus (HSV), CMV, and papovavirus antigens has yielded negative results within microglial nodules that contain HIV-positive mononuclear cells.[94] Wiley et al[95] and Ward et al[98] found HIV infection of endothelial cells; however, this observation has not been confirmed by others. The specific localization of HIV to neurons, astrocytes, and oligodendroglia is uncertain.

Several pathogenetic schemes have been advanced to explain the neurologic dysfunction seen in patients with HIV

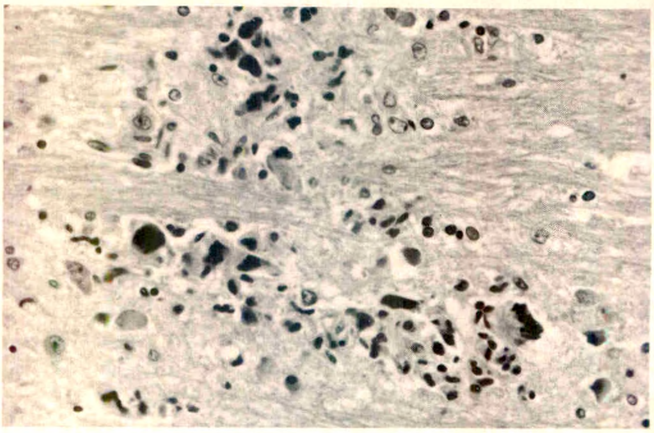

Fig 1.—Human immunodeficiency virus encephalitis. Section of pons shows microglial nodules with multinucleate giant cells (hematoxylin-eosin, original magnification × 120).

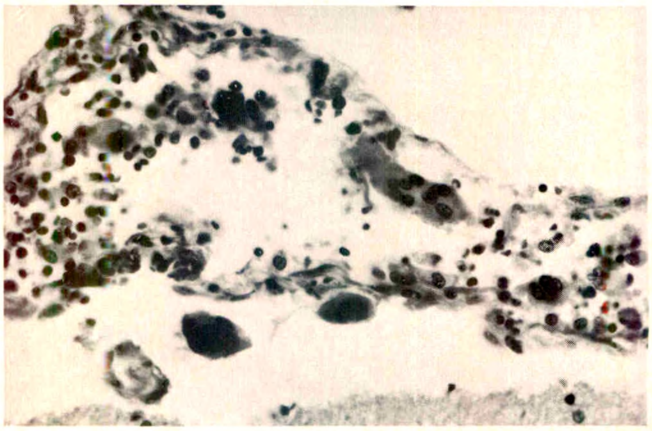

Fig 2.—Human immunodeficiency virus encephalitis. Multinucleate giant cells in subarachnoid space (hematoxylin-eosin, original magnfication × 120).

encephalitis.[37,104] One postulate suggests that HIV-infected monocytes from the circulation are transported across the blood-brain barrier into the CNS, where HIV is stimulated to replicate within monocytes and macrophages. These infected cells could then release monokines or proteolytic enzymes that may be toxic to neurons or glia, or possibly endothelial cells. Another hypothetic mechanism is that the HIV envelope glycoprotein may compete with the neurotrophic factor neuroleukin for binding to certain neurons, thereby causing a shortened survival of affected cells.[105] Human immunodeficiency virus may also involve the cerebral microvasculature, causing an alteration in vascular permeability.[45] Dysfunction of the blood-brain barrier might result from a direct infection of the endothelium by HIV[95] or possibly as an indirect effect of perivascular HIV-infected monocytes on the endothelium. The resulting increased vascular permeability could cause an accumulation of edema fluid within the extracellular space, as well as allow various circulating macromolecules to enter the cerebral parenchyma, both of which could functionally alter or damage neural or glial cells, axons, or myelin.

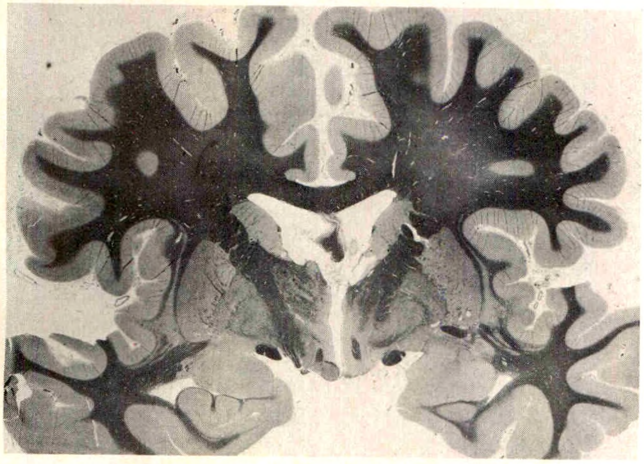

Fig 3.—Human immunodeficiency virus leukoencephalopathy. Whole mount of cerebrum shows pallor of the central white matter (hematoxylin-eosin–luxol fast blue).

Leukoencephalopathy

Apart from the attack on oligodendroglial cells by opportunistic viruses (ie, PML) and the white-matter vacuolar myelopathy discussed below, there is evidence from various laboratories[11,16,44] that suggests that HIV might directly attack the central myelin. The most remarkable examples of a diffuse "demyelinating" lesion that involves large portions of the cerebral white matter are those recorded in the reports of Nielsen et al,[40] Horoupian et al,[39] and Kleihues et al[41] (Fig 3). These authors illustrated myelin-stained sections of the brain at a low magnification in which the intensity of staining is greatly diminished. Microscopically, these sections show a diffuse loss of myelin, a relative sparing of axons, and a paucity of inflammatory cells. Microglial nodules and/or giant cells may be seen within the lesion. There may also be myelin breakdown along the long fiber tracts. We have observed less severe forms of this process characterized by the focal, often angiocentric regions of myelin pallor, sometimes related to aggregates of microglial nodules (Fig 4). Transitions between the focal and more diffuse white-matter lesions may be present. Some of our cases also showed conspicuous axonal damage, with axonal spheroids in the centrum semiovale and in the long fiber tracts. We found prominent changes of the microvasculature in the white matter, including mural thickening, increased cellularity, and enlargement and pleomorphism of endothelial cells. These vascular abnormalities were usually associated with prominent perivascular aggregates of HIV-positive monocytes and multinucleated cells, which often contained hemosiderin pigment. These observations suggested to us a role for altered vascular permeability in the pathogenesis of HIV-associated leukoencephalopathy, as discussed above. Other possible causes for the white-matter degeneration include the destruction of myelin and axons by soluble substances elaborated by HIV-infected monocytes,[106] autoimmune-associated demyelination,[37] and direct attack of HIV on the myelin-forming oligodendrocytes. Some investigators[13,107] have noted alterations in the number and size of

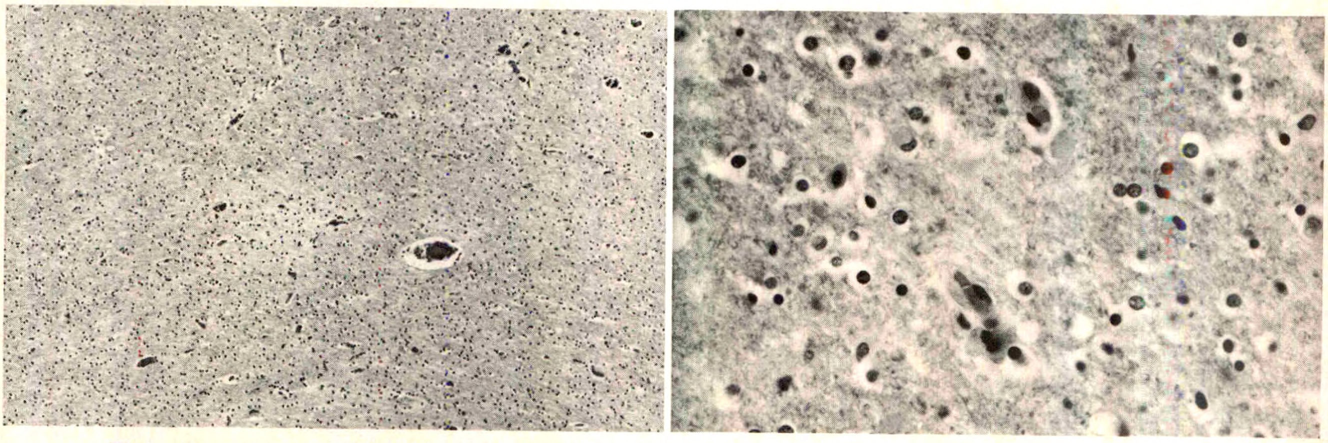

Fig 4.—Human immmunodeficiency virus leukoencephalopathy. Left, Patchy foci of myelin pallor are present in subcortical white matter (luxol fast blue–hematoxlyin-eosin, original magnification × 60). Right, Higher magnification of demyelinated region shows paucity of oligodendroglial cells. Several adjacent blood vessels are surrounded by macrophages (luxol fast blue–hematoxylin-eosin, original magnification × 180).

oligodendrocytic nuclei within areas of myelin pallor; however, the presence of HIV within oligodendroglial cells has not been conclusively demonstrated to date.

Vacuolar Myelopathy

A distinctive disorder of the spinal cord has been described in patients with AIDS who have or do not have associated HIV meningoencephalitis.[21,22] The disorder has been termed vacuolar myelopathy. It is characterized by multiple vacuoles that measure 10 to 50 μm in diameter within the white matter of the posterior and lateral columns of the upper thoracic cord (Fig 5). The histologic appearance is indistinguishable from that of severe subacute combined degeneration. In our experience, the vacuolar change can occasionally be extreme and affect the white matter of much of the cord. There is often no evidence of concomitant gliosis, microglial nodules, giant cells, or a chronic inflammatory reaction. Petito and collaborators[22] believed that there was no relationship between this vacuolar myelopathy and the myelopathies associated with subacute combined degeneration or disorders of vitamin B_{12} metabolism. The role of HIV in the pathogenesis of this lesion is disputed. Several investigators have demonstrated HIV within macrophages localized to the lesions of vacuolar myelopathy, implicating a direct pathogenetic role for the virus.[108,109] However, others found no relationship between the vacuolar lesions and the presence of HIV-infected cells, and they concluded that AIDS-associated vacuolar myelopathy is either not related at all to HIV infection of the spinal cord and brain, or may be a remote or indirect effect of HIV or some other infectious agent.[23,110] The spinal cord has also been reported to be the site of "degenerative" changes that are limited to the corticospinal tracts or the posterior columns. Such changes have been attributed to secondary wallerian degeneration consequent to proximal injury in the pyramidal system or dorsal root ganglia, respectively.[111] Of related interest is the condition known as tropical spastic paraparesis that has been reported in several countries in the Caribbean, Indian Ocean, and South America. Virologic studies suggest human T-cell lymphotropic virus type 1 infection. Pathologic studies are scant, but two recently reported cases[112] have shown a severe lymphocytic meningomyelitis unlike that seen in vacuolar myelopathy.

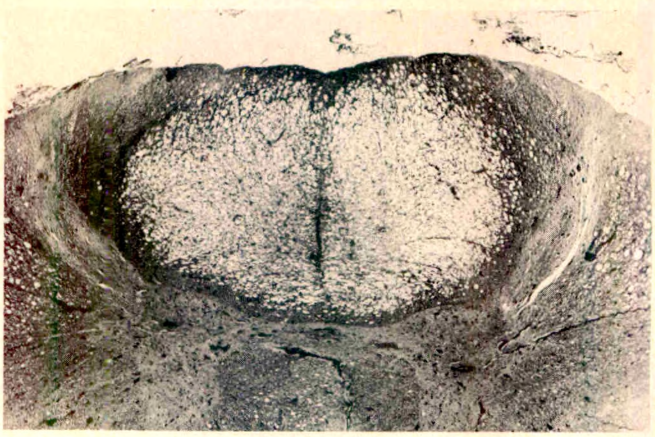

Fig 5.—Vacuolar myelopathy. Section of thoracic spinal cord shows severe vacuolar degeneration in posterior columns and, to a lesser extent, in lateral columns (luxol fast blue–hematoxylin-eosin, original magnification × 12).

Neuropathy

A wide spectrum of cranial and peripheral nerve syndromes have been reported in several series* and in several recent reviews.[24,28,49] In a prospective study of HIV-positive patients described by Gastaut et al[119] the peripheral nervous system was found to be involved in 50 (89%) of 56 patients. The most commonly reported clinical syndromes include acute and chronic inflammatory demyelinating polyneuropathy (in the early stages of HIV infection), distal symmetric polyneuropathy and polyradiculopathy (in the late stages of HIV infection), and mononeuritis multiplex. A sensory neuropathy due to ganglioneuronitis has also been described.[111,120] Many of these clinical syndromes share pathologic features in common, including segmental demyelination, axonal degeneration, and epineurial and endoneurial mononuclear cell inflammation. The etiology of these AIDS-associated neuropathies is multifactorial and often obscure. The neuropathy may be caused by direct HIV infection of the nerve.[28] Human immunodeficiency virus has been cultured from the nerve in some cases of AIDS-associated neuropathy[25,87,94]; however, the virus has not yet been demonstrated within Schwann cells or other peripheral nerve cells by immunohistochemistry or in situ hybridization. In one patient described by Bailey et al,[115] electron microscopy demonstrated retroviruslike particles within the peripheral nerve axoplasm. Necrotizing arteritis has been reported in some cases of AIDS-associated neuropathy.[121,122] In the two patients with vasculitis described by Gherardi et al,[122] HIV was demonstrated in infiltrating mononuclear cells by in situ hybridization. Chaunu and colleagues[27] emphasized the frequency of involvement of the wall of small vessels by mononuclear cells (microvasculitis) in the early stages of the disease. Other infectious agents may cause a neuropathy in some HIV-infected individuals. In the patients described by Bishopric et al,[123] Eidelberg et al,[47] and Behar et al,[124] a Guillain-Barré–like syndrome was related to CMV. Lanska et al[125] described an HIV-positive patient with syphilitic polyradiculopathy. Other possible causes for AIDS-related neuropathies include the following factors: an immune-mediated attack against peripheral nerve components triggered by HIV or other viruses, or by the immunodeficiency state itself; various toxic, metabolic, and nutritional factors; and infiltration of nerves and/or roots by a neoplasm (eg, lymphoma).

Myopathy

Inflammatory myopathy has been the most frequently described skeletal muscle disorder in patients with AIDS or AIDS-related complex.[24,26,50,126-131] The disease is characterized by the subacute onset of proximal weakness, sometimes pain, and an elevated serum creatine kinase level. The histologic findings in these cases have included muscle fiber necrosis and phagocytosis, interstitial infiltration with HIV-positive macrophages, and, in a few cases, cytoplasmic bodies or nemaline rods within myofibers.[24,50,130] To our knowledge, there have been no reports of direct viral invasion of skeletal muscle cells. An inflammatory myopathy has also been described in simian AIDS.[132] Wiley et al[133] recently reported a patient with polymyositis who was seropositive for both human T-cell lymphotropic virus type 1 and HIV. By using in situ hybridization and immunocytochemistry, they observed direct infection of muscle fibers by human T-cell lymphotropic virus type 1 but no evidence of HIV in muscle or inflammatory cells.

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*References 7, 25, 27, 43, 46, 48, 113-118.

The pathogenesis of the inflammatory myopathy is not clear. The hypothesis that skeletal muscle cells are a primary target of HIV has not been borne out by tissue culture or by ultrastructural or viral localization studies. An alternate possibility is that there is an attack on skeletal muscle cells by immune effector cells or that an opportunistic infectious agent alters muscle membranes, rendering them the target of an autoimmune reaction akin to idiopathic polymyositis.[134] In a case of HIV-associated myopathy that we have studied, the presence of structural abnormalities of the microcirculation, immunocytochemical localization of the virus within monocytes around vessel walls, and evidence of altered vascular permeability (extravasation of red blood cells, iron pigment-laden macrophages) suggested that the virus might localize to the vascular bed of skeletal muscle, whether or not the muscle fiber is itself a target cell. Such regional distribution of viruses has been proposed as one possible mechanism of viral tropism.[135]

Recently, two groups have reported an acute toxic myopathy with myoglobulinuria developing in patients with AIDS who were treated with zidovudine (azidothymidine [AZT]).[136,137] These cases seem to illustrate a different pathologic process from the inflammatory myopathy described above. Some patients with AIDS or AIDS-related complex may experience proximal muscle weakness with a normal creatine kinase level; a muscle biopsy specimen showed severe type II fiber atrophy.[24] This condition may be related to poor nutrition, rapid weight loss, prolonged bed rest, or a remote effect of malignancy.

Vascular Lesions

Embolic cerebral infarction and hemorrhages in various sites (intraparenchymal, subarachnoid, and epidural and subdural) have been reported.[7,52] Several cases of CNS vasculitis in patients with AIDS or AIDS-related complex have appeared in the literature. Yankner et al[51] described a patient from the Massachusetts General Hospital, Boston, with CNS granulomatous vasculitis associated with HIV isolation from brain tissue and cerebrospinal fluid. Scaravilli et al[138] reported a patient with AIDS who had multiple cerebral infarcts in association with chronic basal meningitis and a vasculitis histologically similar to that in the previous case. Vinters et al[139] described a patient with AIDS-related complex who had evidence of a systemic necrotizing vasculitis that most severely affected the CNS, with hemorrhagic necrosis of the spinal cord and cauda equina. Nonvasculitic proliferative lesions that affect smaller-diameter vessels within the leptomeninges[140] and cerebral parenchyma[52] have also been described. Calcific vasculopathy that involves the basal ganglia has been described in children with AIDS (see below). We have observed abnormalities of the cerebral microvasculature with evidence of altered vascular permeability in cases of HIV meningoencephalitis and leukoencephalopathy (see discussion above). As yet, there has been no unequivocal demonstration of HIV (by electron microscopy, in situ hybridization, and/or immunocytochemistry) within intrinsic vascular cells in any of the AIDS-associated vasculopathies.

AIDS-Dementia Complex

Many clinical reports[3,4,43,44,141] refer to a progressive deterioration of intellectual function—"dementia"—in patients with AIDS. This cognitive disorder usually occurs well into the course of the illness, but sometimes it may be the presenting manifestation of the disease. Motor abnormalities, ataxia, bladder and bowel incontinence, and seizures can also be present. Computed tomography of the brain has shown cortical atrophy and ventricular dilatation. Abnormalities of the cerebral white matter may be seen by magnetic resonance imaging.[142] Postmortem neuropathologic study of patients with these clinical and radiologic abnormalities has shown a wide spectrum of abnormalities that range from HIV encephalitis to disseminated infections and combinations of these. Thus, it is likely that the pathogenesis of the cognitive impairment may differ in affected individuals. In some of our patients with radiologic evidence of brain "atrophy" and ventricular dilatation, neuropathologic examination has demonstrated myelin and axon loss in the subcortical and central white matter rather than degeneration of the cerebral cortex.

Pediatric Disease

The neuropathologic abnormalities of AIDS in children differ somewhat from those in adults. These have been discussed in several recent reviews.[31,32,107,143] The most frequently reported neuropathologic abnormality in pediatric AIDS is calcification of the basal ganglia and deep cerebral white matter. In some cases, progressive calcification has been demonstrated by computed tomographic scans. Histologically, this lesion consists of mineral deposits (calcium, iron) affecting both large and small vessels, ischemic neurons, microglial nodules, and large destructive lesions associated with lymphoma or vascular occlusion.[32] The next most frequent finding is HIV meningoencephalitis/leukoencephalopathy as described in adults (see above). The spinal cord in these cases may show secondary wallerian degeneration of the pyramidal tracts; however, vacuolar myelopathy has not yet been reported. Opportunistic infections of the CNS, including toxoplasmosis, CMV, PML, and cryptococcal meningitis, are relatively rare in infants and children. The most common focal CNS abnormalities reported by Dickson et al[32] were lymphomas and cerebrovascular lesions. Myopathies and peripheral neuropathies have not been reported in children.

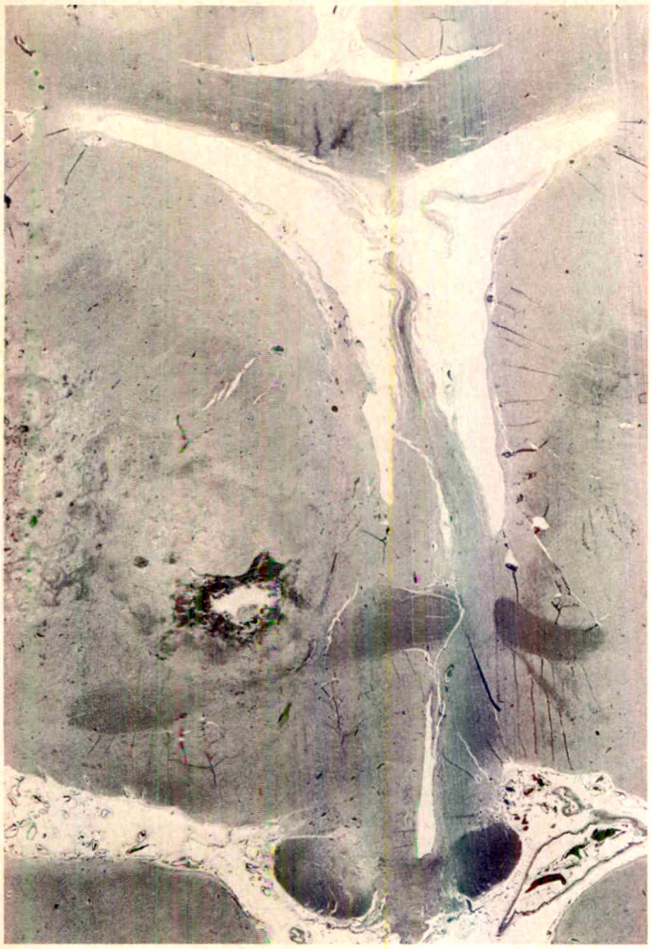

Fig 6.—Cerebral toxoplasmosis. Whole mount shows acute *Toxoplasma* abscess with necrotic center in anterior putamen (hematoxylin-eosin, original magnification × 1.5).

OPPORTUNISTIC INFECTIONS

Toxoplasmosis

Toxoplasma gondii encephalitis has been reported by a number of investigators. The encephalitis tends to be clinically more fulminant and pathologically more devastating than in the nonimmunocompromised host.[57] It is considered to be the most common cause of focal brain lesions in patients with AIDS. Histologic studies of CNS toxoplasmosis complicating AIDS, based on both surgical and postmortem material,[56,57] have shown that the lesions are frequently multiple and most often involve the cerebral cortex, subcortical white matter, and deep gray nuclei and, less often, the cerebellum and brain stem (Fig 6). Rarely, the spinal cord may be involved.[144,145] The lesions may be grouped into three histologic types based on the degree of tissue reaction: necrotizing, organizing, and chronic. All of these may coexist in the same patient. Microscopically, the earliest lesions—necrotizing abscesses—consist of foci of necrosis with variable petechial hemorrhages surrounded by a mixed and variable degree of acute and chronic inflammation, macrophage infiltration, and vascular proliferation (Fig 7, left). The small arteries that surround the abscesses may show marked intimal proliferation or even frank vasculitis with fibrinoid necrosis and thrombosis.[146] Hematoxylin-eosin–stained sections reveal abundant free tachyzoites typically located at the periphery of the necrotic foci. Encysted bradyzoites may also be present. Both the free tachyzoites, as well as the encysted bradyzoites, may be demonstrated in paraffin-embedded sections by immunocytochemical staining with the use of commercially available anti–*Toxoplasma* antisera (Fig 7, right). Organizing abscesses consist of large well-demarcated areas of coagulation necrosis that are surrounded by lipid-laden macrophages. Cysts and free tachyzoites may also be found adjacent to these lesions, although they may be considerably reduced in number if therapy has been effective. Chronic abscesses consist of small (usually <0.5 cm in diameter) cystic spaces that contain small numbers of lipid-laden and occasional hemosiderin-laden macrophages with surrounding gliosis. Organisms may be difficult to detect near these lesions. Recently, a diffuse "encephalitic" form of cerebral toxoplasmosis has been reported in AIDS in which microscopic examination has shown widespread microglial nodules associated with *Toxoplasma* cysts or free tachyzoites within brain parenchyma.[147]

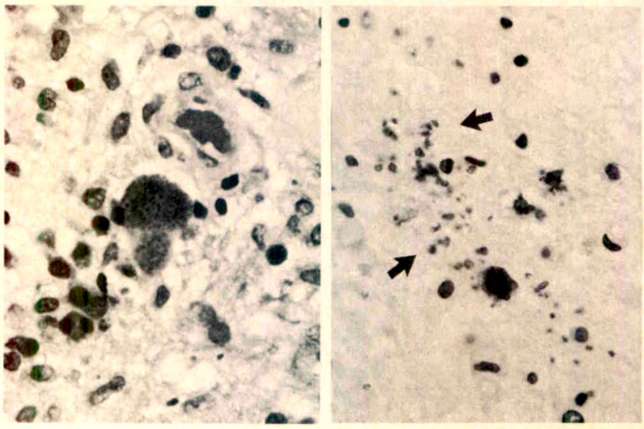

Fig 7.—Cerebral toxoplasmosis. Left, *Toxoplasma* cysts containing bradyzoites (hematoxylin-eosin, original magnification × 180). Right, Immunostaining of cyst and free tachyzoites (arrows) using anti–*Toxoplasma* antibody (peroxidase-antiperoxidase, original magnification × 180).

Fungi, Mycobacteria, and Spirochetes

Fungi.—Among the pathologic fungi, *Cryptococcus neoformans*, *Candida* species, *Aspergillus* species, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis* have all been reported in decreasing order of frequency.[58] The histopathologic characteristics of the fungal meningoencephalitis caused by these agents are indistinguishable from those that occur in the non-AIDS population, except that the lesions tend to be more extensive, the destructive process is more necrotizing, and the inflammatory response is less prominent. Infection with *C neoformans* characteristically affects the basal leptomeninges that become thickened and opaque by reactive connective tissue and may obstruct the outflow of cerebrospinal fluid from the foramina of Luschka and Magendie, giving rise to hydrocephalus. Sec-

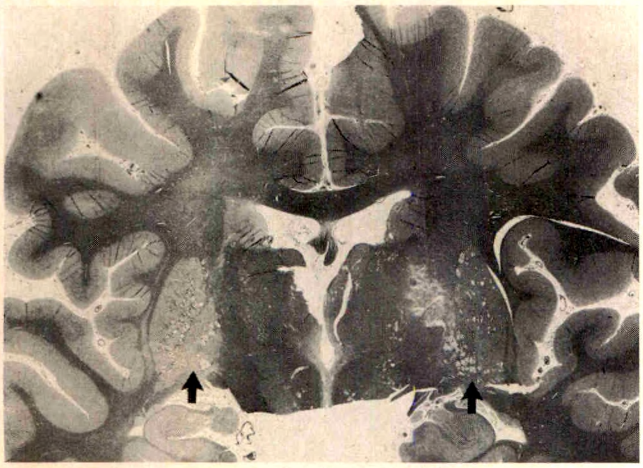

Fig 8.—Cryptococcal meningitis. Whole mount of cerebrum shows characteristic "soap bubble" lesions (enlarged Virchow-Robin spaces) in base of brain, especially in basal ganglia (arrows) (mucicarmine).

tions of the brain disclose a gelatinous material within the subarachnoid space and small cysts within the brain, especially at the base in the distribution of the lenticulostriate arteries (Fig 8). Microscopically, the intraparenchymal lesions consist of aggregates of organisms that are usually present within expanded perivascular Virchow-Robin spaces associated with a minimal or absent leukocytic or glial response (Fig 9). Meningeal infiltrates contain variable numbers of chronic inflammatory cells and fibroblasts admixed with the yeasts in which capsules can be well demonstrated with the mucicarmine or periodic acid–Schiff reactions.

***Mycobacterium avium-intracellulare*.**—Infection of the meninges and brain with *M avium-intracellulare* has been reported infrequently in patients with AIDS.[16,19] The inflammatory response tends to be less pronounced than in *Mycobacterium tuberculosis* and is described as consisting of foamy macrophages and chronic inflammation without distinctive tubercles but with a clear-cut demonstration of the organisms by special staining techniques. The response to tuberculosis chemotherapeutic agents has been poor.

***Treponema pallidum*.**—Patients with HIV infection are at an increased risk for the development of neurosyphilis.[59,148] The rate of progression and the severity of the disease appear to be accelerated in patients with AIDS, suggesting that the alteration in cell-mediated immunity seen in HIV infection may alter the natural course of syphilis.[148] The CNS involvement by *T pallidum* may be manifested as asymptomatic infection, acute syphilitic meningitis, meningovascular syphilis, and, rarely, direct parenchymal invasion of the brain. In the series reported by Katz and Berger,[59] almost half of the patients with neurosyphilis also had syphilitic eye disease. An exceptionally severe necrotizing encephalitis with massive treponemal invasion of the brain (so-called quaternary neurosyphilis) has been reported in a patient with HIV infection.[149,150]

Viral Infections

CMV.—The most frequently recorded opportunistic viral pathogen that causes meningoencephalitis in patients with AIDS is CMV.[38,66] The virus tends to localize in the ependyma and the subependymal regions of the brain. The earliest evidence of the destructive process can often be demonstrated adjacent to the ependymal surface where the striking large intranuclear inclusion bodies are evident[60] (Fig 10). Immunocytochemical and in situ hybridization techniques have demonstrated that morphologically normal but latently infected cells can be found well beyond the site of cells that show viral inclusions.[65-67] A postmortem study of CMV encephalitis in AIDS from New York by Morgello and coworkers[66] identified five major categories of CMV-associated lesions in decreasing order of frequency: microglial nodules, isolated inclusion-bearing cells, focal parenchymal necrosis, necrotizing ventriculoencephalitis, and necrotizing radiculomyelitis. Microglial nodules and CMV inclusions were the most common lesions in this series. The microglial nodules occurred with variable frequency and were most often encountered in the subcortical gray matter; however, only relatively few contained CMV inclusion-bearing cells. Isolated inclusion-bearing cells, unac-

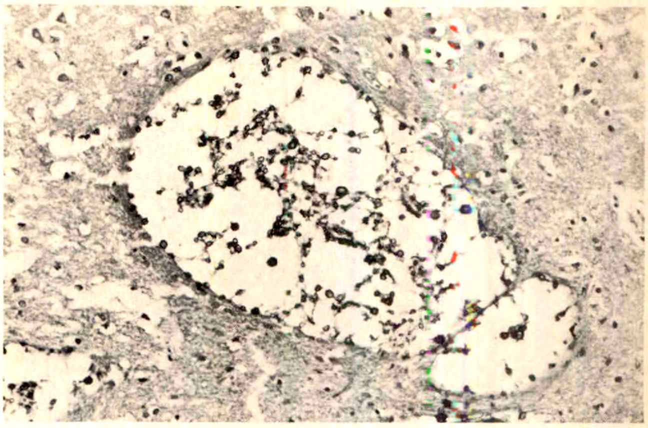

Fig 9.—Cryptococcal meningitis. Enlarged perivascular (Virchow-Robin) space contains numerous cryptococcal organisms (periodic acid–Schiff, original magnification × 60).

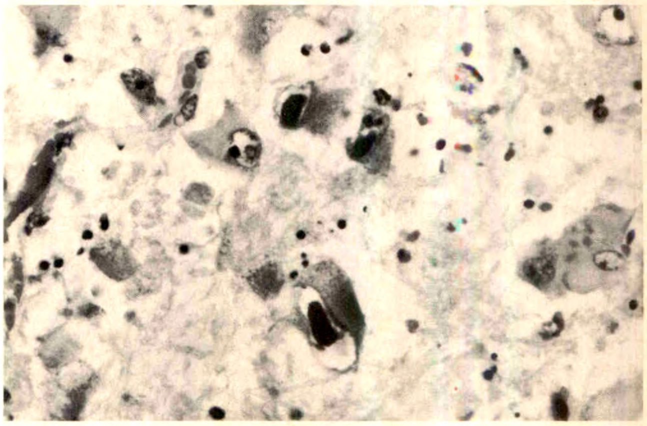

Fig 10.—Cytomegalovirus encephalitis. Section from periventricular region shows enlarged glial cells containing typical cytomegalovirus intranuclear inclusions (hematoxylin-eosin, original magnification × 180).

companied by microglial nodules or inflammatory infiltrates, were seen in half of their cases, and could be identified in the capillary endothelium, astrocytes, and neurons. Some patients with AIDS can have infection of the brain by both HIV and CMV[151]; thus, it is possible that the microglial nodules observed in these cases could be caused by either virus.

HSV.—Herpes simplex virus encephalitis can occur in immunosuppressed patients with an incidence and pathologic picture similar to that seen in immune competent individuals.[152,153] The brain macroscopically shows necrosis that predominantly involves regions of the limbic system, namely, the temporal and inferior frontal lobes, insulae, and cingulate gyri. Acute cases may show swelling, congestion, and petechial or sometimes larger hemorrhages in affected areas. The characteristic histologic features include the following types: vascular changes (congestion, petechial hemorrhages, fibrinoid necrosis, and thrombosis of small vessels); acute eosinophilic necrosis of neurons; Cowdry type A intranuclear inclusions; and perivascular mononuclear cell inflammation in the gray and white matter and leptomeninges. Polymorphonuclear leukocytes can also be seen in areas of severe involvement. The Cowdry type A inclusion bodies have an eosinophilic homogeneous appearance and may be separated from the nuclear margin by a narrow clear zone; they can occur in both neurons and glia. Cowdry type A inclusions may not always be present in a given case of HSV encephalitis; hence, their absence should not necessarily exclude a diagnosis of this condition. The immunoperoxidase method is a particularly useful technique for the identification of HSV antigen in paraffin-embedded sections.[154] The viral antigen can be demonstrated in both the nucleus and cytoplasm of neurons and glia (oligodendrocytes, astrocytes, and, rarely, ependymal cells), and may be seen in both acutely injured and normal-appearing cells. Cowdry A inclusions also stain for HSV antigen. Herpes virions can be demonstrated by electron microscopy; however, this appears to be a relatively less sensitive diagnostic method.[155] The herpesvirus genome has been detected by in situ hybridization in paraffin-embedded brain biopsy specimens of HSV encephalitis.[156] Viral cultures in most cases of herpes simplex encephalitis yield HSV type 1; however, several cases of encephalitis caused by HSV type 2 have been reported in immunocompromised patients.[70,157,158]

To date, HSV encephalitis in its classic form appears to be uncommon in patients with AIDS.[107] However, cases of combined HSV-CMV ventriculo-encephalitis[66] and HSV type 2 necrotizing myelitis[63,69,70,159] have been reported in patients with AIDS.

Varicella-Zoster Virus (VZV).—Herpes zoster accounts for 12% of all herpesvirus infections in patients with AIDS.[159] The VZV-associated disorders that involve the CNS in AIDS include the following types: encephalomyelitis,[11,71,160] herpes zoster ophthalmicus,[161,162] trigeminal encephalitis,[73] leukoencephalitis,[72] and cerebral vasculopathy.[72] The pathologic features of VZV infection consist of viral inclusion bodies in the nuclei of neurons within the gasserian or dorsal root ganglia. The VZV nucleic acid has been shown by in situ hybridization to be present in the neurons of normal human ganglia, supporting the notion of latency and reactivation of the virus at a time of reduction of immune defenses.[163] The inflammatory process may extend into the meninges, dorsal roots, dorsal horns, spinal cord, and brain stem.[164] Rostad et al[162] described a patient with herpes zoster ophthalmicus who pathologically showed transsynaptic spread of VZV through the visual system. Cases of VZV encephalitis may show edema, perivascular or leptomeningeal chronic inflammation, perivascular demyelination, fibrin thrombi in vessels, necrosis, hemorrhage, and, in some instances, viral inclusions in glial cells, and, rarely, endothelial cells.[165-167] Rare patients may have a leukoencephalitis characterized by multifocal demyelinating lesions that resemble PML, with VZV inclusions in glial cells and neurons surrounding the lesions.[71,72,168] The VZV-associated vascular disease is most commonly seen in patients with herpes zoster ophthalmicus but can also be seen in other forms of VZV infection. Both granulomatous arteritis[169,170] and a noninflammatory vasculopathy associated with the presence of VZV antigen in vascular walls[72,171] have been described.

PML.—The disorder named PML or Richardson's disease was initially described by Aström et al[172] and Richardson[173] as a disorder of the white matter that occurs in patients with defective immune responses. The most commonly observed predisposing conditions have been chronic lymphoproliferative, myeloproliferative, or granulomatous diseases. From the outset, it had been hypothesized that the disorder might result from an atypical viral infection, and in 1965, to our knowledge, Zu Rhein and Chou[174] published the first ultrastructural images of oligodendroglial nuclei that contained profiles that resembled papovavirus. Virus isolation followed in 1971,[175] which, throughout the years, has been almost always identified as the JC papovavirus. Progressive multifocal leukoencephalopathy has been reported in patients with AIDS.[74-76,175-179] The incidence of PML in patients with AIDS may be as high as 3.8%.

Pathologically, the lesions consist of patches of irregular ill-defined granular destruction of the white matter, ranging in size from a few millimeters to extensive involvement of an entire lobe of the brain or cerebellar hemisphere (Fig 11).

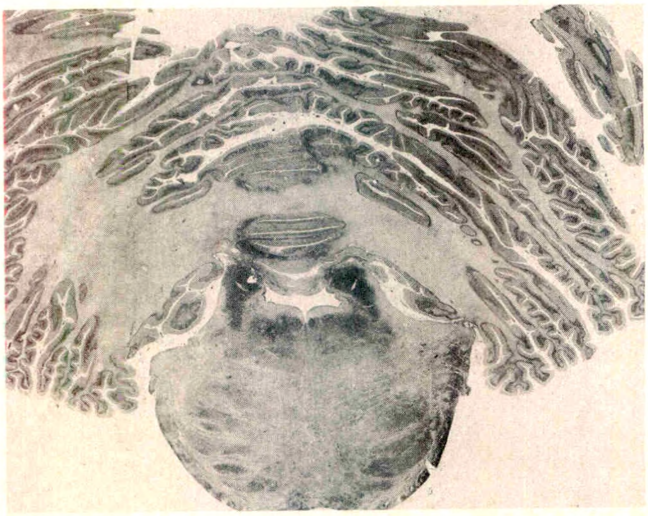

Fig 11.—Progressive multifocal leukoencephalopathy. Whole mount of cerebellum and pons shows extensive confluent demyelination in cerebellar white matter and basis pontis (hematoxylin-eosin–luxol fast blue, original magnification × 1.2).

Whole-brain histologic sections, stained for myelin, demonstrate the multifocal lesions as having a "moth-eaten" appearance. In patients with AIDS, the extent and severity of the PML lesions may be greater than that observed in non-AIDS immunocompromised individuals.

Microscopically, the typical lesion consists of a patch of demyelination in the center of which are many fat-laden macrophages and a few axons. At the edge of the lesion are greatly enlarged oligodendroglial nuclei in which the chromatin is replaced by glassy amphophilic viral inclusion material. These oligodendrocytes can be shown to contain viral antigen by the immunoperoxidase method (Figs 12 and 13, left) and by in situ hybridization.[180] Additional characteristics include the presence of bizarre, giant astrocytic nuclei (Fig 13, right) and occasional perivascular and subarachnoid infiltrates with lymphocytes and plasma cells. Wiley et al[76] demonstrated that macrophages that infiltrate the demyelinating PML lesions in patients with AIDS may also be infected by HIV.

NEOPLASMS

Lymphomas

Primary CNS lymphoma has been reported with increasing frequency in patients with AIDS.[6,77,78,80,81,181-183] It is second only to toxoplasmosis as a cause of focal cerebral masses in AIDS.[184] The older literature referred to these tumors as microgliomas; however, during the past 10 years, it has been recognized that the neoplastic cell is the same as that seen in non-Hodgkin's lymphoma when it occurs outside the CNS. These tumors are frequently multicentric and involve the cerebrum, brain stem, and cerebellum in decreasing order of frequency. The CNS lymphomas in patients with AIDS often show extensive coagulative necrosis, which radiologically may resemble other necrotizing brain lesions, such as toxoplasmosis. Extension to the ventricles and/or subarachnoid space has been recognized, thereby providing a useful diagnostic test through cytologic examination of the cerebrospinal fluid. The histologic classification of primary CNS lymphomas in patients with AIDS can be difficult because of the pleomorphic mixture of both neoplastic and nonmalignant lymphoid cells present in many cases.[81] The neoplastic lymphoid cells have ranged from small, noncleaved cells with multiple nucleoli to the large cells of immunoblastic lymphoma. Marker studies have shown reactions consistent with a B-cell origin in most cases. The neoplasm tends to be infiltrative and shows a perivascular arrangement of tumor cells at the edge of the lesion (Fig 14). This reaction is highlighted with the reticulin stain that demonstrates a characteristic concentric network of silver-positive fibers. In general, tumor behavior is similar to that observed in the non-AIDS population[182]; however, one recent study has emphasized a greater tendency toward multicentricity.[81]

The high incidence of non-Hodgkin's B-cell lymphomas in patients with AIDS is common to other acquired and naturally occurring immunologic disorders, including transplant recipients and patients with autoimmune disease and immunoblastic lymphadenopathy. The lymphomas in these conditions are most likely the result of the clonal evolution of neoplastic cells from a polyclonal pool of abnormally stimulated B lymphocytes in conjunction with impaired T-cell surveillance.

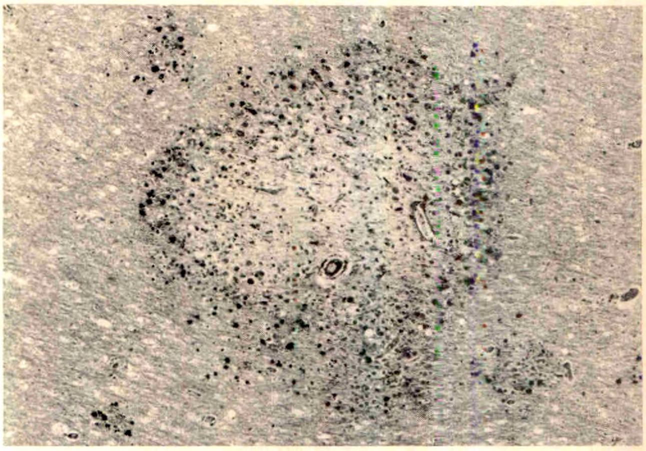

Fig 12.—Progressive multifocal leukoencephalopathy. Demyelinating lesion shows enlarged virus-infected oligodendroglial cells located predominantly at periphery of lesion. In this section, the oligodendroglial nuclei have been stained with an immunocytochemical stain for papovavirus (peroxidase-antiperoxidase–luxol fast blue, original magnification × 12).

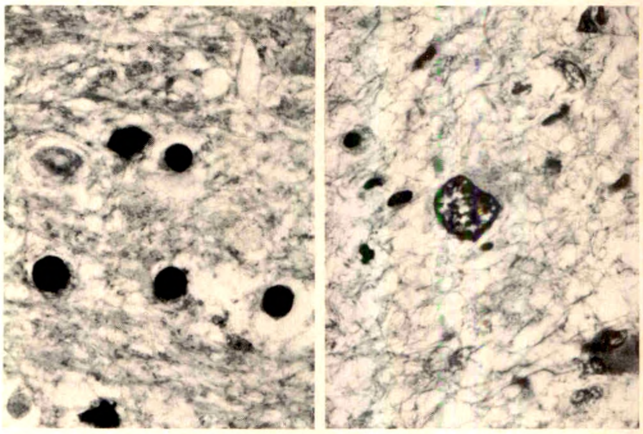

Fig 13.— Progressive multifocal leukoencephalopathy. Left, Oligodendroglial nuclei stained with antibody for papovavirus (peroxidase-antiperoxidase–luxol fast blue, original magnification × 180). Right, Demyelinating lesion contains bizarre giant astrocyte (hematoxylin-eosin–luxol fast blue, original magnification × 180).

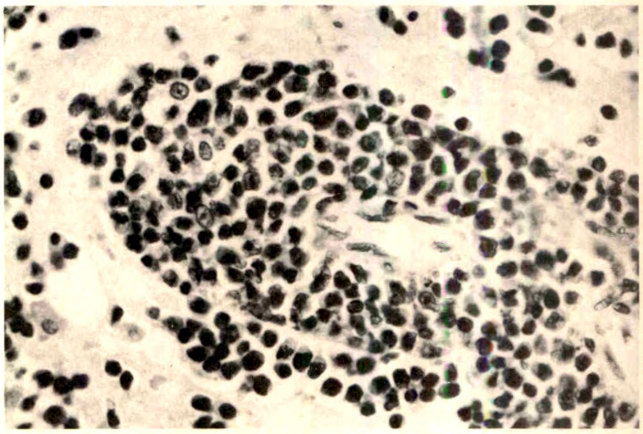

Fig 14.—Primary central nervous system lymphoma (large-cell type). Note the perivascular distribution of lymphoma cells (hematoxylin-eosin–luxol fast blue, original magnification × 180).

The detection of Epstein-Barr virus sequences by in situ hybridization in CNS lymphomas occurring in patients with AIDS has suggested a possible pathogenetic role for this virus.[185,186]

Spread of systemic lymphoma to the CNS has been recorded with greater frequency in the AIDS than in the non-AIDS population.[79,187-189] Secondary lymphomatous infiltrates primarily involve the leptomeninges, cranial and spinal nerves, and paraspinal epidural space in contrast to primary CNS lymphomas, which tend to involve the brain parenchyma. The systemic lymphomas in patients with AIDS have demonstrated an unusually high prevalence of high-grade neoplasia and a poor response to therapy.

Kaposi's Sarcoma

Kaposi's sarcoma infrequently metastasizes to the CNS. This subject has been reviewed by Gorin et al.[82]

COMMENT

Despite the many publications on the neurologic and neuropathologic manifestations of AIDS, there are still many important problems that need further study. The biology of HIV infection in the nervous system is poorly understood. There has been speculation that CNS HIV may be a specific subgroup of the virus. The HIV isolates from the central nervous system have been shown to differ in many respects from peripheral blood and lymph node isolates.[190] Furthermore, it has been recently demonstrated that the amount of unintegrated HIV DNA is greatest in the CNS tissue of patients with HIV encephalitis.[191] There is a close correlation between the presence of unintegrated viral DNA and cytopathic changes. Another major question is when and how HIV enters the nervous system. Does the virus invade the brain early in the course of the disease and remain latent in fixed macrophages only to be reactivated by subsequent opportunistic infections, a neoplasm, or other trigger? Conversely, could HIV infection of the brain be a late manifestation of systemic dissemination of the virus? The pathogenesis of many of the neuropathologic lesions observed in patients with AIDS remains uncertain. The cause of one of the most devastating AIDS-related neurologic syndromes—AIDS dementia—is unknown.

Autopsy studies on patients with AIDS will be extremely useful in providing suitable tissues for continued virologic research and to clarify unsolved clinicopathologic questions. In addition, continued complete postmortem examinations will be helpful in defining the neuropathology of related infections, such as HIV type 2 and human T-cell lymphotropic virus type 1, the effects of treatment, and the identification of new or perhaps as yet unrecognized lesions.

D. L. Walker, PhD, University of Wisconsin Medical School, Madison, provided the antiserum with regard to Fig 12.

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Vascular Transformation of Lymph Node Sinuses

A Process Displaying a Spectrum of Histologic Features

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• Nonneoplastic vascular lesions in lymph nodes have been infrequently reported. These lesions are believed to be rare and display histologic features ranging from minimal changes associated with vasodilation to vascular proliferative lesions resembling Kaposi's sarcoma. The spectrum of histologic features observed in these cases appears to be the result of the extent and duration of regional lymphatic and/or venous obstruction. We describe two such cases, one presenting in a 66-year-old black woman, after undergoing radical mastectomy for treatment of invasive ductal carcinoma of the breast following a breast biopsy; the other presenting in a 66-year-old black man with obstruction of the subclavian vein. We consider these cases worthy of review, both from a diagnostic standpoint and in the differential diagnosis of vascular neoplasms of lymph nodes, especially Kaposi's sarcoma.

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Vascular transformation of lymph node sinuses due to venous obstruction was first described in humans by Haferkamp and colleagues in 1971. They described vascular changes in three patients, two of whom had associated regional malignant disease. Since then, few references to these lesions have been made in the literature. In 1975, Fayemi and Toker[2] reported four cases of a proliferative, benign vascular process encountered in lymph nodes of women aged 50 to 70 years. Three of their patients were undergoing radical mastectomies for invasive mammary ductal carcinoma; one patient had Hodgkin's disease. They designated these lesions as *nodal angiomatosis*. Lott and Davies[3] described two hypervascular lymph node lesions that they termed *hemangiomatoid* and *pan-nodal vasodilation*. The hemangiomatoid lesions were noted in single lymph nodes that were excised with axillary lymph node groups for treatment of mammary carcinoma. Their pan-nodal vasodilatation cases were most commonly found in patients with ulcerative colitis, or carcinoma of colonic or mammary origin. Although not mentioned in the title of this article, they also described a "miscellaneous" lesion encountered in the hilar areas of an axillary lymph node removed in the treatment of carcinoma of the breast.

REPORT OF CASES

CASE 1.—A 66-year-old black woman had undergone a left breast biopsy procedure 3 weeks previously that revealed an invasive ductal carcinoma 3.5 cm in diameter. The current material was a left radical mastectomy specimen. Results of the remainder of the physical examination and laboratory findings were unremarkable.

CASE 2.—A 66-year-old previously healthy black man from Jamaica who had lived in the United States for the past 28 years presented with a 2-week history of swelling of the left arm. On physical examination, a firm, movable 1- to 2-cm left supraclavicular lymph node was noted as well as a tight, swollen left upper extremity. Results of the remainder of the physical examination and laboratory findings were within normal limits. A venogram revealed thrombosis of the left subclavian vein. The palpable left supraclavicular lymph node was excised, along with some adjacent connective and lymphoid tissue.

PATHOLOGIC FINDINGS

The specimen from patient 1 was a modified radical mastectomy that contained axillary lymph nodes without metastatic disease. Notably, however, there was invasive ductal carcinoma in

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the lower portion of the axillary adipose tissue. This appeared to be in contiguity with residual primary mammary neoplasm that was situated in the upper outer quadrant of the breast. In the subcapsular sinusoidal spaces of axillary lymph nodes, there was a proliferation of flat to spindled endothelial cells with round to oval, distended lumena that contained sparse red blood cells (Fig 1). No mitotic activity or cellular pleomorphism was noted. There was associated capsular fibrosis, mainly in the areas of vascularity. Focally, these endothelial-lined spaces formed a sievelike pattern (Fig 2). The remainder of the lymph node architecture was unremarkable, and there were no hilar thromboses. Hemosiderin was absent throughout.

The specimen from patient 2 consisted of multiple supraclavicular lymph nodes, the largest measuring 1 cm in diameter. Numerous vessels and associated fibrous tissue distorted the entire architecture of the subcapsular and sinusoidal regions, and replaced a large portion of the lymphoid tissue (Fig 3). The vessels ranged in size from slitlike spaces lined by plump endothelial cells to channels up to 2 mm in diameter. The largest vessels contained red blood cells, fibrin, and fluid that resembled lymph. These were most prominent in the hilar regions. Areas with small vessels and associated fibrous tissue focally simulated Kaposi's sarcoma (Fig 4). There was red blood cell extravasation in these areas, however, no hemosiderin, hyaline globules, or cellular atypia was noted. Areas similar to those seen in patient 1 were also noted (Fig 5).

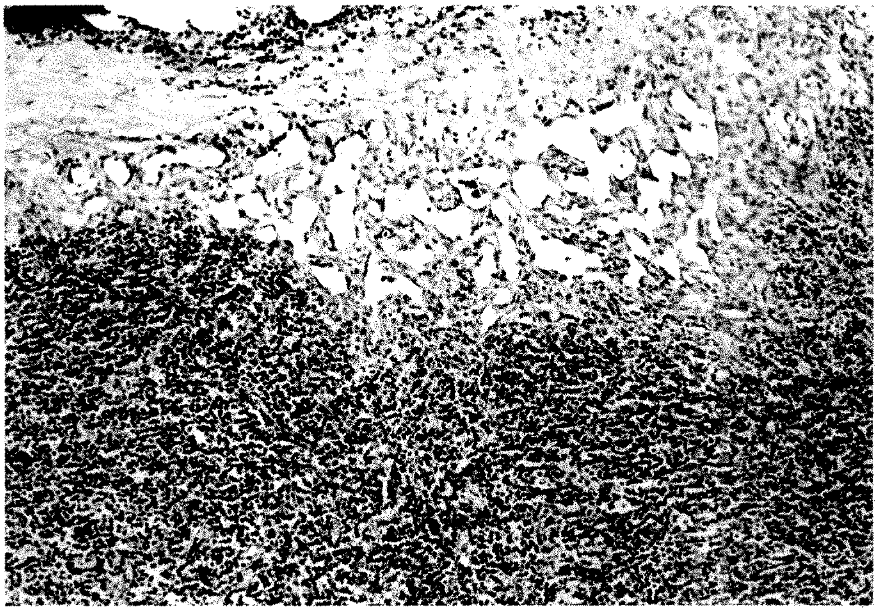

Fig 1.—Axillary lymph node with subcapsular proliferation of vascular tissue, with focal associated fibrosis.

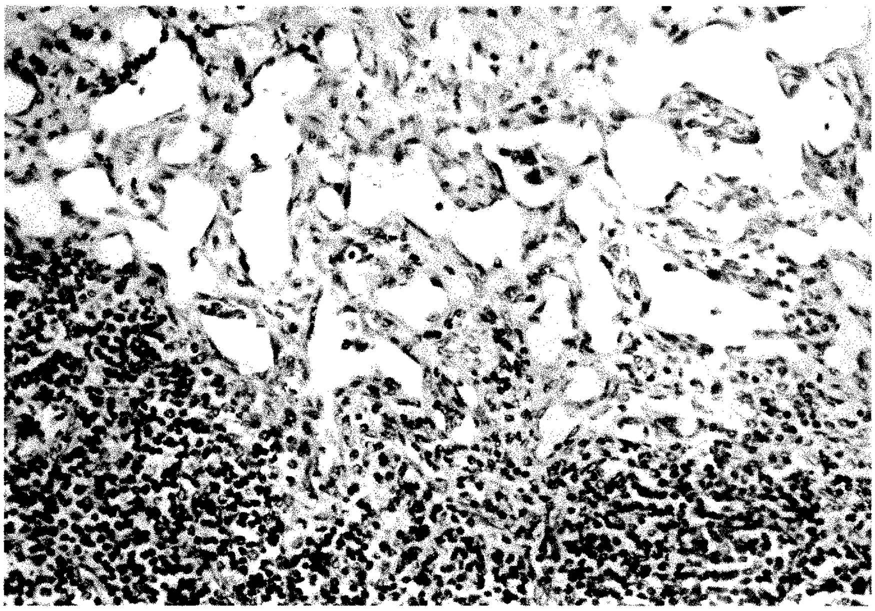

Fig 2.—Proliferation of vascular and connective tissue, sievelike pattern. The vascular spaces are lined by flattened and spindled endothelial cells.

COMMENT

Vascular transformation of lymph node sinuses was initially reported by Haferkamp et al.[1] One of their patients had acute obstruction of the superior vena cava with swelling and cyanosis of the head and neck and a 1-cm mediastinal lymph node. Another "peanut-sized" painful lymph node was present in the axilla of an otherwise asymptomatic 22-year-old woman; one patient had a 3-cm retroperitoneal lymph node associated with a pelvic and retroperitoneal malignant lymphoma. Lymph nodes from their three cases exhibited extensive thrombotic obliteration of hilar and perinodal veins.[1] The sinusoidal architecture was replaced by a network of anastomosing vascular channels, some of which were large and tortuous; others closely resembled small capillaries. No cytologic atypia was present, and there was a varying amount of associated fibroplasia. They interpreted these histologic findings as the sequelae of severe venous obstruction, resulting in increased venous pressure and shunting of blood into lymphatic channels. This also apparently precipitated a reorganization of the lymph node sinuses, with the subsequent capillarylike formations. Haferkamp et al[1] noted that the earliest changes, seen 10 days after the onset of symptoms, consisted of recently organized hilar thromboses and early fibroplasia. The lesions of longer dura-

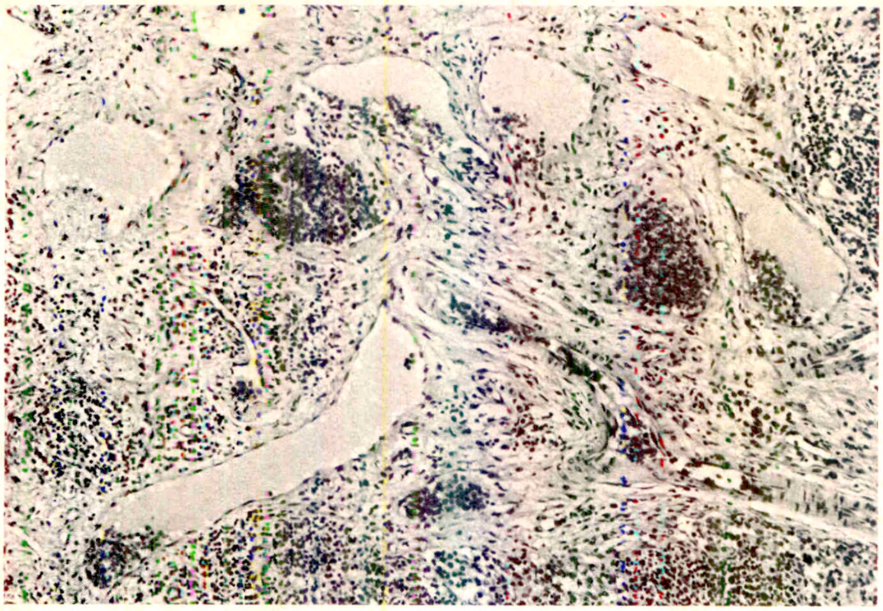

Fig 3.—Extensive replacement of supraclavicular lymph node parenchyma by vascular and connective tissue. Note associated lymphoid atrophy, and focal dilation of some medullary vessels.

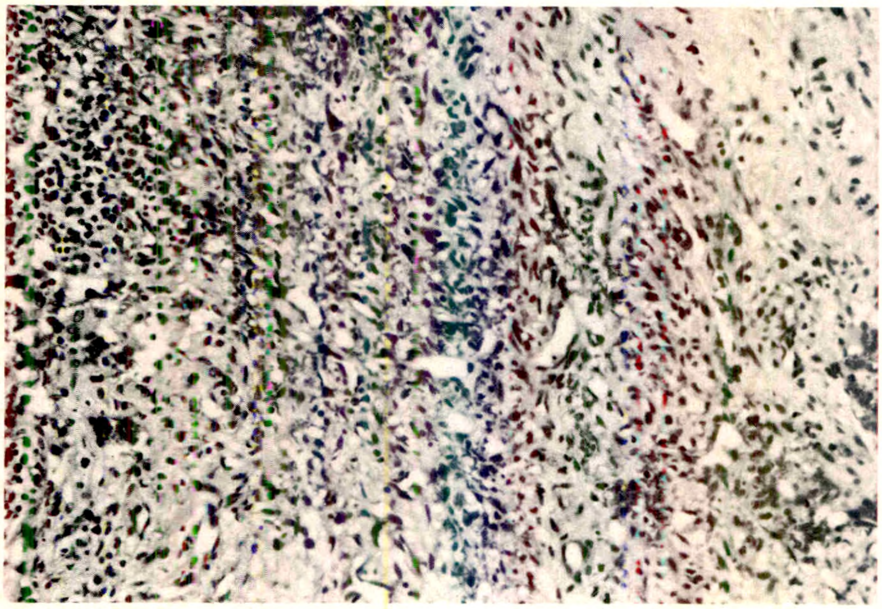

Fig 4.—Relatively more solid area with closely packed spindle-type endothelial cell proliferation, focal fibrosis, and red blood cell extravasation, simulating Kaposi's sarcoma.

tion (5 weeks and later) displayed more marked vascularized and hyalinized fibrosis with associated lymphoid atrophy and sclerosis of the hilar veins. The histologic features seen in our patient 2, obtained 2 weeks after the onset of symptoms, closely resembled these latter cases. Although no thrombosis of the nodal or perinodal hilar veins was observed, there was a large thrombus in the left subclavian vein noted on venographic studies.

Regions studied in patient 2 (Fig 3) also resembled the entity described by Lott and Davies[3] as a hemangiomatoid lesion of the lymph node. These lesions consisted of a plexus of vessels of varying caliber extending into the lymph node parenchyma from the hilar regions. These changes were noted in single lymph nodes in axillary lymph node dissection specimens obtained from two patients with carcinomas of the breast. These authors also reported a second lesion that they designated as pan-nodal vasodilation. This process, consisting of a proliferation of vessels, apparently small veins, was present diffusely in the medullary and cortical regions as well as in perinodal connective tissue. Features of these lesions were also similar to those noted in patient 2 (Fig 3). Most of the patients with this lesion had carcinoma of the breast or colon; some had ulcerative colitis. They described a third lesion that showed an apparent proliferation of vascular and smooth-muscular tissue in the hilar regions of four axillary lymph nodes, also from a woman with mammary carcinoma. Although not postulated by the authors,[3] all of these latter features may have been secondary to lymphatic and/or venous obstruction. It is interesting that a significant number of their patients had carcinomas in the vicinity of the lymph nodes examined.

Fayemi and Toker[2] reported a vascular proliferative lesion of lymph nodes that they designated as nodal angiomatosis. In their cases, a benign vascular proliferation in the cortical and medullary lymph node regions was noted. Although the lymph node architecture was primarily intact without lymphoid atrophy, the vascular proliferation in the subcapsular sinusoidal regions was striking. Small, round to oval lumena were lined by flattened to cuboidal endothelial cells without cytologic atypia or mitotic activity. These features closely resembled those seen in patient 1 (Figs 1 and 2) and also focally in patient 2 (Fig 5). This histologic picture is identical to that illustrated by Schnitzer.[4] However, he designates these lesions as vascular transformation of lymph node sinuses, as do Dorfman and Warnke.[5] Bedrosian[6] presented two additional cases of nodal angiomatosis and noted the similarity of these lesions to those reported as vascular transformation of sinuses by Haferkamp et al.[1]

Steinmann et al[7] was able to experimentally produce vascular changes in lymph nodes. They produced congestion in cervical lymph nodes of rabbits by occluding nodal veins, efferent lymphatics, or both. Complete occlusion of

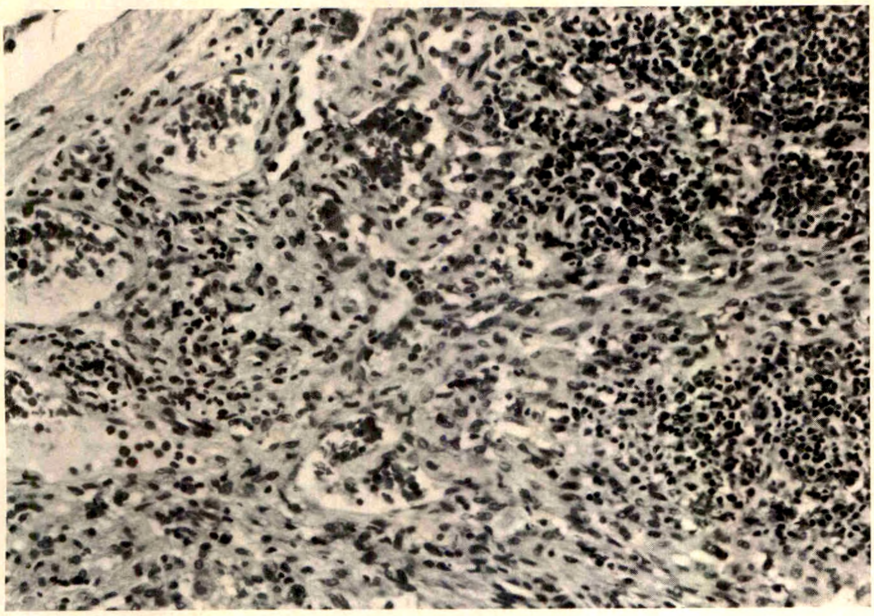

Fig 5.—Subcapsular region of supraclavicular node, demonstrating vascular proliferation similar to that depicted in the axillary lymph nodes of patient 1 (Fig 1).

both lymphatics and veins led to total necrosis (infarction) of nodal parenchyma. This corresponded to changes noted in superficial lymph nodes described by Davies and Stansfeld,[8] which they believed were due to venous occlusion. Incomplete occlusion of the veins combined with complete occlusion of the lymphatics or complete occlusion of the lymphatics alone resulted in vascular transformation of lymph node sinuses, as previously described by Haferkamp et al.[1] This feature was noted as early as 7 days after the occlusion(s). In this early stage of venous sinus thrombosis, proliferation of subendothelial tissue along with blood capillary proliferation was noted, initially most prominent along the capsular side of the marginal sinus. Subsequently, the sinuses were transformed into a framework of channels resembling blood capillaries. These changes corresponded to those noted in our cases (Figs 1, 2, and 5) as well as those described by Fayemi and Toker.[2] Complete or incomplete occlusion of veins combined with complete occlusion of lymphatics also resulted in a pronounced thickening of the capsule, sclerosis of the medullary sinuses, depletion of nodal lymphocytes, and cavernous dilation of some hilar vessels. These changes corresponded to those noted in patient 2 (Fig 3) and those previously documented by Haferkamp et al.[1] Occlusion of veins alone caused moderate thickening of the capsule and nodal parenchymal sclerosis, but no vascular transformation. Vascular transformation did not develop without occlusion of the efferent lymphatics.

Lucke et al[9] detailed similar lesions in nine domestic cats, which they termed *plexiform vascularization of lymph nodes*. These lesions occurred in the cervical and inguinal regions of otherwise healthy animals and consisted of capillary vasoproliferation and lymphoid atrophy. They were reportedly found in over 5% of surgical biopsy specimens of cat lymph nodes over a 5-year period.

Rousselet et al[10] recently described a benign vascular proliferative lymph node lesion that they interpreted as a reactive healing process, and which histologically resembled exuberant granulation tissue. Clinically, this patient had serologic evidence of *Toxoplasma gondii* infection. Along with the spindled endothelial cells were areas of cortical coagulative necrosis. This latter feature separates this lesion from the others.

In contradistinction to these cases are several relatively infrequent vascular neoplasms of lymph nodes. There are three reported cases describing lymph node hemangiomas.[11-13] These lesions are relatively well circumscribed, as opposed to the diffuse nature of the vascular lesions seen with lymphatic or venous obstruction. They seem to present more frequently in the pediatric age group. The nodal hemangioma reported by Almagro et al[12] was from a patient who also had intestinal angiodysplasia and a hemangiopericytoma of the oral mucosa.

Two cases of primary hemangioendotheliomas of lymph nodes have been reported[14,15]; one with a spindle and histiocytoid (epithelioid) pattern.[15] The latter lesion lacked well-defined vascular spaces and was studied with electron microscopy and immunohistochemical stains. Endothelial differentiation was demonstrated using both modalities (Weibel-Palade bodies and factor VIII-related antigen positivity in tumor cells, respectively). These lesions closely resembled the examples of histiocytoid hemangiomas and epithelioid hemangioendotheliomas described by Rosai and colleagues.[16]

Recently, Suster and Rosai[17] described a peculiar intranodal hemorrhagic spindle cell tumor. These lesions were characterized by highly vascularized interlacing fascicles of spindle cells with a rim of surrounding lymph node. Immunohistochemical stains revealed actin, myosin, and vimentin positivity. Electron-microscopic studies demonstrated features indicative of myofibroblastic and smooth-muscle differentiation. Focally stellate areas of collagen in an "amianthoid" pattern were noted.

One case originally presented as a primary hemangioma of the lymph node was later reclassified as an example of a metastatic angiomatoid malignant fibrous histiocytoma.[18] This case was that of a 28-year-old woman with an axillary metastasis from a primary tumor situated in the elbow region. These neoplasms usually display significant cellular pleomorphism with up to three mitoses per high-power field.

Another important vascular neoplasm of lymph nodes seen in increasing numbers and usually in association with immunosuppression is Kaposi's sarcoma. Although lymph nodes are usually secondarily affected by Kaposi's sarcoma, this entity still probably represents the most common vascular neoplasm arising in lymph nodes.[19-27] These lesions display a spindle cell proliferation with slitlike spaces, extravasated red blood cells, and hemosiderin. Hyaline globules are present intracellularly and extracellularly. The spindle cells usually exhibit at least a mild degree of cytologic atypia and are somewhat larger than the usual type of endothelial cells. Larger vessels or vessels with more open lumena are not considered a diagnostic component of this neoplasm. Kaposi's sarcoma can present in a solid, nodular, and well-circumscribed fashion in lymph node tissue, but can also infiltrate lymph node sinuses in a pattern similar to that seen in patient 2 (Fig 4). Localized sinusoidal deposits can also be seen. Both the intranodal hemorrhagic spindle-cell tumor with "amianthoid" fibers described by Suster and Rosai[17] and the nodal benign vascular proliferation reported by Rousselet et al[10] focally resembled nodal Kaposi's sarcoma; the latter case was originally diagnosed as such. Some cases of lymphadenopathic Kaposi's sarcoma are apparently not associated with immunosuppression.[28]

We believe the spectrum of features exhibited in both the previously reported nonneoplastic cases (with the exception of Rousselet and coworkers' case[10]) and in our cases represent sequelae of lymphatic and/or venous obstruction. We agree with Kasznica et al[13] that these lesions are "proliferative-reactive" in nature. In our opinion, the lesion described as nodal angiomatosis[2,6] and vascular transformation of sinuses[1] represent opposite ends of this spectrum of changes that are associated with partial or total obstruction of lymphatic and/or venous channels, respectively. The studies by Haferkamp et al,[1] Fayemi and Toker,[2] and Lott and Davies[3] detail different phases of this process. We prefer the designation of vascular transformation of sinuses for these changes because it more accurately denotes the microscopic features seen in these cases and describes their nonneoplastic character. It is increasingly important to differentiate these lesions from Kaposi's sarcoma.

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Ultrastructural Morphometry in the Diagnosis of Sézary Syndrome

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• Blood specimens from 87 patients and control subjects were prepared for electron microscopy and subjected to ultrastructural morphometric evaluation by using a computerized planimeter. A statistical comparison of means indicated that patients with Sézary syndrome could be distinguished from normal subjects and patients with reactive lymphocytosis, by using mean nuclear perimeter and form factor values. The lymphocytic nuclei from patients with infectious mononucleosis were more lobated on visual inspection than those from normal subjects; the difference in mean form factor values was statistically significant. The simple histogram method was most discriminatory and distinguished patients with Sézary syndrome from patients with other types of lymphoid leukemias and reactive lymphocytosis, including infectious mononucleosis. The histogram method could not, however, distinguish patients with Sézary syndrome from patients with T-cell chronic lymphocytic leukemia and Japanese T-cell leukemia. The use of bivariate graphic displays (plotting nuclear size and shape measurements) placed the lymphoid cells of the various types of lymphoproliferative disorders into distinct morphometric domains. Computerized morphometric techniques may, therefore, be of greater value when the range of possible diagnoses is large.

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Leukemias, presenting as small lymphocytes with abundant heterochromatin, can provide a diagnostic challenge for hematopathologists at the light microscopic level.[1-6] Difficulties in the diagnosis are underscored by the fact that some "well-differentiated" leukemic cells often share many morphologic and immunophenotypic similarities with their normal or reactive counterparts.[2,4] This cytologic mimicry between nonneoplastic and malignant cells results from the fact that the dysregulation of differentiation in lymphoid malignancies can be extremely subtle.[7] The Sézary syndrome (SS),[8,9] which represents the leukemic phase of a convoluted,[10] small-cell, T-cell lymphoma of skin,[11] can present such a diagnostic challenge. Sézary cells, when present in small numbers in the peripheral circulation,[4] can be confused with reactive lymphocytes,[12-16] including viral-induced lymphocytosis. When present in higher numbers, Sézary cells can be confused morphologically, with other types of chronic leukemias, such as chronic lymphocytic leukemia (CLL),[1,3] plasma cell leukemia,[13] and the "buttock cell"[17] that characterizes the leukemic phase of a nodal-based lymphoma (LPL).[18-22]

It is now recognized that the light microscopic identification of Sézary cells in the peripheral blood of patients with mycosis fungoides (MF) carries a poor prognosis.[23-25] A quantitation of these cerebriform mononuclear cells was deemed necessary,[23-25] since cerebriform mononuclear cells have been identified in the blood of healthy individuals and can appear Sézary-like.[26] To diagnose SS, Vonderheid et al[23] used a value of greater than 15% circulating atypical lymphocytes with cerebriform nuclei, and Sausville et al[25] used a value of greater than 20% lymphocytes with atypical nuclear convolutions. A more accurate identification of circulating Sézary cells is therefore paramount for an accurate diagnosis and prognosis. Ultrastructural morphometric studies from our laboratory,[27-31] and those of others,[32-38] have proved the usefulness of quantitation in distinguishing MF from reactive lymphoid infiltrates of skin and MF from convoluted B-cell lymphomas.[30] Our previous morphometric studies also indicated that "mathematically defined" malignant Sézary cells could not be produced by in vitro stimulation using a series of mitogens and antigens,[28] and that the vast majority of reported Sézary-like cells, present in benign conditions, could not be included in the unequivocal malignant range,[28] which was morphometrically defined from skin biopsy specimens from patients with MF by using the sensitive[39] shape analytic formula $(4\ \pi A[\text{area}]/P[\text{perimeter}]^2)$ that defines form factor[40]

The purpose of the present ultrastructural morphometric study was to determine the best discriminatory nuclear parameters in circulating lymphocytes that are most useful in the diagnosis of SS. A novel approach to the evaluation of leukemias, in general, by using bivariate graphic displays to establish morphometric domains, is also described.

PATIENTS AND METHODS

Patient Selection

The following clinical groups that contained a total of 87 cases were included in the

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study: 20 normal subjects (10 female and 10 male); 15 cord blood specimens; 10 children with reactive lymphocytosis (RL) (9 cases were virally induced); 5 adults with viral-induced lymphocytosis (3 cases of Epstein-Barr virus [positive] and 2 cases of cytomegalovirus [positive]); 8 patients with SS; 10 patients with B-type CLL; 4 patients with LPL; 1 patient with T-cell CLL; 1 patient with Japanese T-cell leukemia; 4 patients with natural killer cell (NK) lymphoproliferative disorder (LPD); 2 patients with common acute lymphocytic leukemia; 2 patients with T-cell acute lymphocytic leukemia; 2 patients with hairy-cell leukemia; and 1 patient with cold agglutinin disease. All patients with benign and malignant LPDs, for which there were glutaraldehyde-fixed venous blood specimens available, were included in the study, representing a range of "stimulated" lymphocytes and other forms of leukemia, some of which have grooved or clefted nuclei by light microscopy. Blood was initially drawn under the guidelines of our institution's informed consent procedures. All charts were reviewed, and a final diagnosis was obtained; diagnoses were based on a combination of clinical findings, biopsy results, routine light and electron microscopy, and immunophenotyping (when available).

The eight patients with SS all had biopsy-proved MF, by using the combined results from light microscopy, immunohistochemistry, and quantitative electron microscopy as previously described.[30] The biopsy specimens were taken from skin lesions described clinically as exfoliative erythrodermatitis (three cases), atopic dermatitis (two cases), and erythroderma (three cases).

Preparation of Cells for Electron Microscopy

Blood specimens from 87 patients and control subjects were spun in Wintrobe tubes, and the buffy coat layer was fixed in situ with 3% glutaraldehyde in 0.1-mol/L phosphate buffer. The buffy coat plug was removed, postosmicated, dehydrated, and embedded in Spurr's epoxy resin.

Ultrastructural Morphometric Techniques

Selection of Cells for Analysis.—One hundred nuclei from each case were photographed under the electron microscope at a scope magnification of ×3600, and all negatives were printed on 20×25-cm sheets of photographic paper. In the nonleukemic cases, only mature-appearing lymphocytes with abundant heterochromatin were selected for analysis. Leukemic cells were identified by their characteristic chromatin patterns and cytologic detail. In those cases where the leukemias were defined by light microscopy as small cells with abundant heterochromatin (eg, CLL, SS, and NK LPDs), all lymphoid cells with a mature chromatin pattern were photographed for morphometric evaluation.

Tracing Procedures.—Each electron micrograph was placed on the sensitized tablet of a manually operated plainmeter image analyzer (MOP-3, Carl Zeiss Inc, New York, NY),[41] and the nuclear contour was manually traced by using a sensitized pen. The circumference of the nuclear membrane that forms a pseudoinclusion was not traced.

Computations.—Perimeter (P), area (A), and FF values were obtained for each nucleus. The FF ($4\pi A/P^2$) is a measurement that bears an inverse linear relationship to the degree of deviation from a circle. The FF value of a perfect circle is 1.0. Values less than 1.0 indicate various degrees and forms of nuclear irregularities,[39] with the lowest values indicating the greatest deviation from a circle. The methods and rationale used for measuring nuclear contours based on only a nuclear outline (which does not include enclosed cytoplasmic areas) have been previously reported.[28] When a plane of section cuts through a lobated nuclear so that pseudodisconnected lobes appear in the resultant photograph, the FF value for the entire nucleus is obtained by weighing the FF value for each lobe based on the respective square micron area, as previously described.[27]

Statistics.—The mean ± SD of each ultrastructural parameter was determined for each case. The SD and CV was used as a measure of inherent cellular variability within individual cases. A mean group value ± SD was then obtained for selected control and leukemic groups. The mean group SD and CV were used as a measure of heterogeneity among cases within each group. The means were compared by using Student's *t* test. The difference between two means was considered statistically significant if $P<.05$.

Morphometric Domains.—A morphometric domain is a two-dimensional cluster plot that allows one to compare experimental groups visually by using two different variables simultaneously. The rationale and practical aspects of the use of multivariate analysis is beyond the scope of this article, but readers should refer to previously published studies on the topic.[42-44] In the present study, the mean nuclear FF and mean nuclear area (A) for each case were used as x,y coordinates in a bivariate graphic display.[30,45-48] The morphometric domains were determined by generating a series of polygons. The polygons were drawn by joining the furthest points in the cluster to form a "wrap" around the entire cluster, as previously described.[47,48]

Histogram Technique (Percentage of Lymphocytes With 0 to 12 Sharply Angled Nuclear Invaginations [NIs]).

A sharply angled NI is defined as an indentation of the nucleus that forms an angle by at least one side of the indentation with the nuclear surface that is 90° or less. Criteria used for evaluating NIs were as previously described.[27] Briefly, NIs that were not scored as "sharply angled" included shallow indentations that subtended an angle greater than 90° with the surface and curved profiles where surface tangent lines could not be accurately drawn. Readers who want to use this histogram method on their own photographs should refer specifically to the diagram shown in the article by Payne and colleagues,[27] which describes acceptable vs unacceptable NIs by using a series of tangent lines. The number of sharply angled NIs has been given for each of the 27 nuclei shown in Figs 1 through 3 of the present study (see figure legends).

The number of NIs was determined by scoring consecutive lymphocytes under the electron microscope, while traversing the buffy coat from top to bottom in adjacent grid windows, until a total of 100 lymphocytes were scored. A histogram that showed the percentages of lymphocytes having 0 to 12 sharply angled NIs was then prepared.

RESULTS

Ultrastructural Appearance of Normal, Reactive, and Neoplastic Lymphocytes

The ultrastructural variability of the nuclei shown in Figs 1 and 2 characterized the normal and reactive populations of lymphocytes found in non-leukemic subjects. Leukemic populations, on the other hand, are less variable, and are characterized by populations of cells with similar types of nuclear shapes and/or chromatin patterns (Fig 3). For example, the leukemic nuclei found in B-cell CLL have smooth nuclear contours (Fig 3G), those in Japanese T-cell leukemia are lobated (Fig 3C), the nuclei of Sézary cells are highly convoluted or cerebriform in appearance (Fig 3A), and cells in T-cell CLL can have several sharply angled NIs (Fig 3B). Many of the leukemic cells bear a striking similarity to certain lymphocytes found in normal or reactive blood. Compare, for example, a nucleus from B-cell CLL (Fig 3G) with its possible normal counterparts in Figs 1A, 1B, 2A, and 2B, a Sézary nucleus (Fig 3A) with Figs 1G and 2D, Japanese T-cell leukemia (Fig

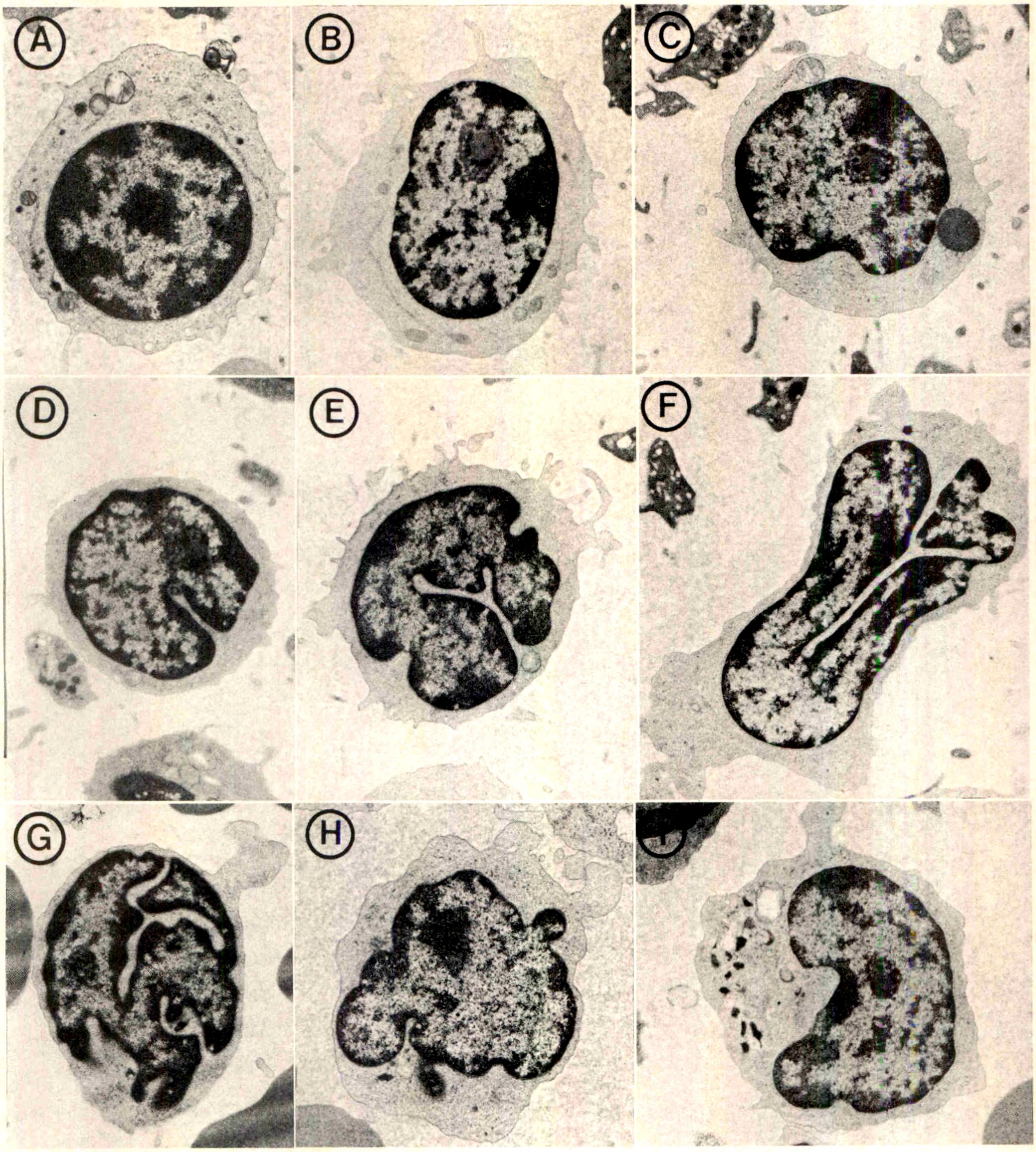

Fig 1.—A through I, Ultrastructural variability of lymphocytic nuclei obtained from the peripheral blood of normal subjects (uranyl acetate–lead citrate, ×6400). A, Round contour; no sharply angled nuclear invaginatons (NIs). B, Oval shape; no sharply angled NIs. C, Indentation of nuclear membrane that is not sharply angled; no sharply angled NIs. D, Contour containing one sharply angled NI. E, Contour containing three sharply angled NIs. F, Stalked nuclear lobe (nucleotesimal); one sharply angled NI. G, Sézary-like cell with a convoluted appearance; seven sharply angled NIs. H, Petal formation, clover-leafing or lobated appearance; no sharply angled NIs. I, Natural killer–like cell (no sharply angled NIs); note the presence of cytoplasmic granules near the hof region of the nucleus. These granules were identified as containing parallel tubular arrays at higher magnification.

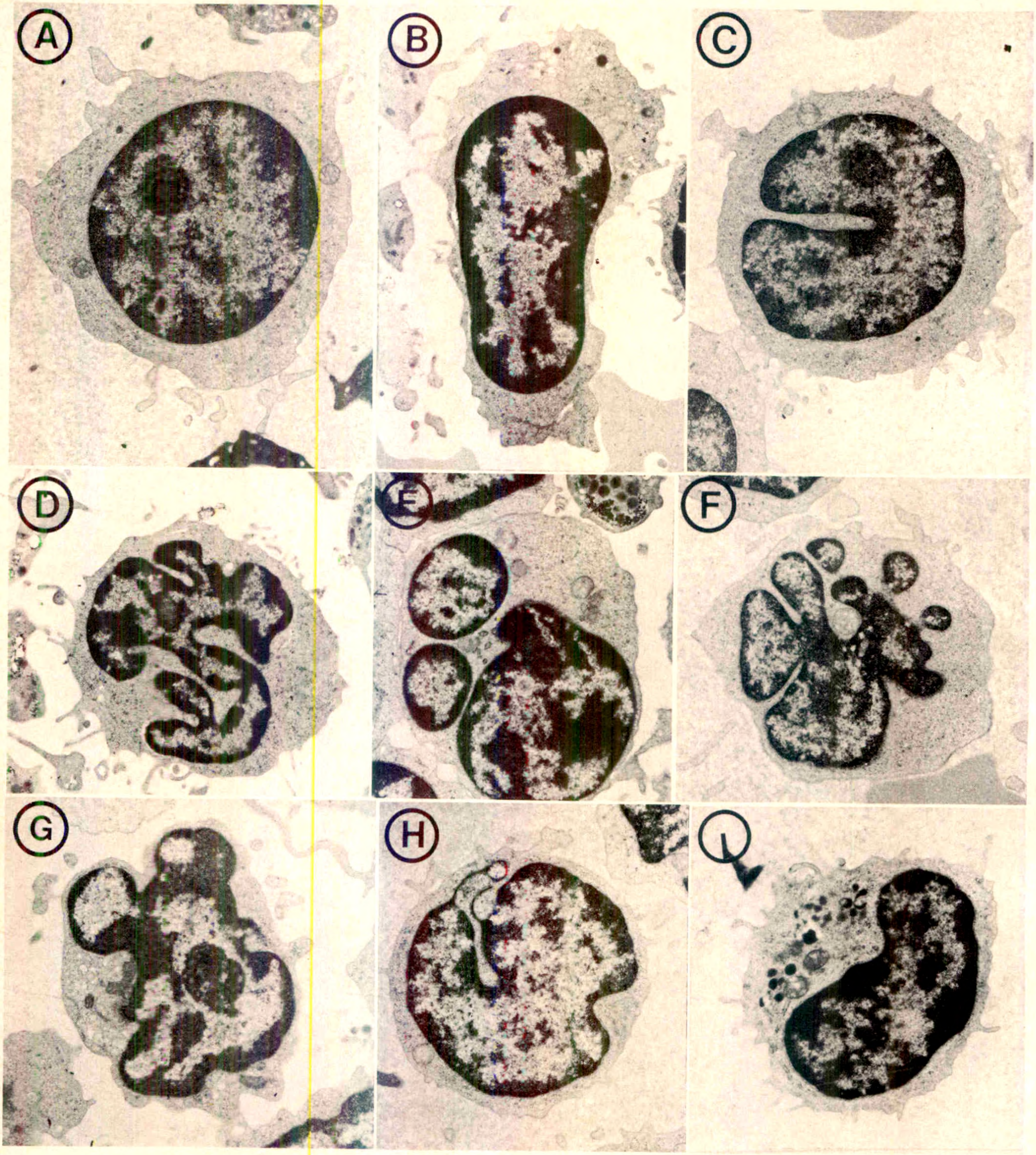

Fig 2.—A through I, Ultrastructural variability of lymphocytic nuclei obtained from the peripheral blood of patients with reactive lymphocytosis (uranyl acetate–lead citrate, ×6400). A, Round contour; no sharply angled nuclear invaginations (NIs). B, Oval shape; no sharply angled NIs. C, Contour containing one sharply angled NI. D, Sézary-like cell with a convoluted appearance; four sharply angled NIs. E, Three pseudodisconnected lobes; no sharply angled NIs. F, Petal formation, clover-leafing or lobated appearance; two sharply angled NIs. G, Lobated appearance; one sharply angled NI. H, Stalked nuclear lobe (nucleotesimal) and fingerlike projection; no sharply angled NIs. I, Natural killer–like cell shows slight indentation of nuclear contour; no sharply angled NIs. Note the presence of cytoplasmic granules near the hof region of the nucleus.

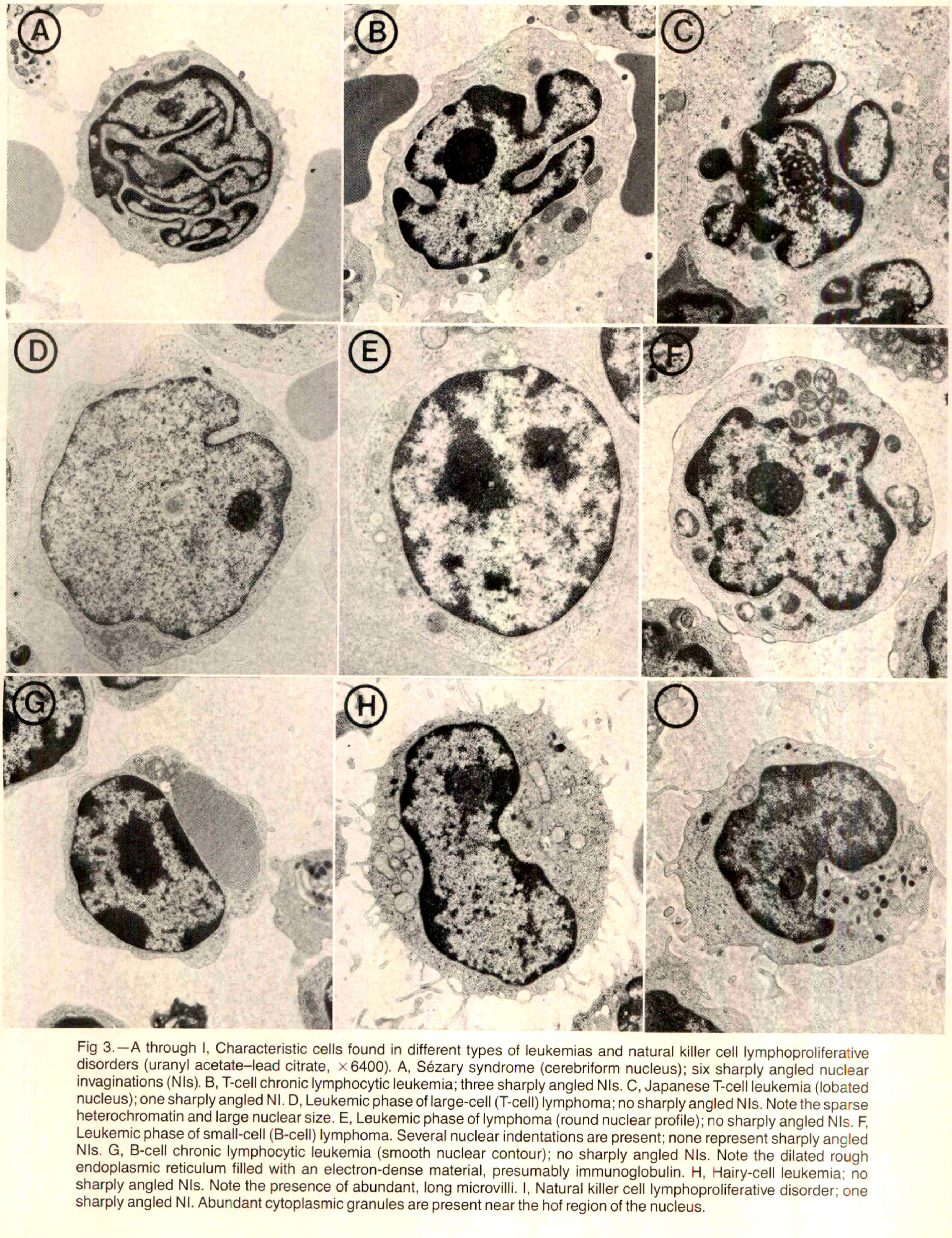

Fig 3.—A through I, Characteristic cells found in different types of leukemias and natural killer cell lymphoproliferative disorders (uranyl acetate–lead citrate, ×6400). A, Sézary syndrome (cerebriform nucleus); six sharply angled nuclear invaginations (NIs). B, T-cell chronic lymphocytic leukemia; three sharply angled NIs. C, Japanese T-cell leukemia (lobated nucleus); one sharply angled NI. D, Leukemic phase of large-cell (T-cell) lymphoma; no sharply angled NIs. Note the sparse heterochromatin and large nuclear size. E, Leukemic phase of lymphoma (round nuclear profile); no sharply angled NIs. F, Leukemic phase of small-cell (B-cell) lymphoma. Several nuclear indentations are present; none represent sharply angled NIs. G, B-cell chronic lymphocytic leukemia (smooth nuclear contour); no sharply angled NIs. Note the dilated rough endoplasmic reticulum filled with an electron-dense material, presumably immunoglobulin. H, Hairy-cell leukemia; no sharply angled NIs. Note the presence of abundant, long microvilli. I, Natural killer cell lymphoproliferative disorder; one sharply angled NI. Abundant cytoplasmic granules are present near the hof region of the nucleus.

Table 1.—Mean Group Values of Ultrastructural Morphometric Nuclear Parameters From Normal Subjects and Patients With RL and Leukemias*

	Mean ± SD					
			RL			
Parameter	Normal Subjects	CB	Children	Adults	SS	B-CLL
Nuclear perimeter, μm	16.17 ± 0.94	19.37 ± 1.00	18.98 ± 1.51	18.01 ± 1.23	21.33 ± 2.32	16.22 ± 1.38
SD nuclear perimeter, μm	2.77 ± 0.34	4.28 ± 0.71	3.25 ± 0.45	3.31 ± 0.72	5.77 ± 1.20	2.13 ± 0.50
CV nuclear perimeter	17.2 ± 1.9	22.0 ± 2.9	17.1 ± 2.0	18.2 ± 2.8	27.0 ± 3.7	13.1 ± 2.6
Nuclear area, μm^2	14.61 ± 1.33	15.83 ± 1.48	17.22 ± 2.10	15.26 ± 1.02	14.61 ± 2.12	17.20 ± 2.64
SD nuclear area, μm^2	2.64 ± 0.20	3.14 ± 0.41	2.57 ± 0.53	3.12 ± 0.48	3.37 ± 0.67	3.21 ± 0.73
CV nuclear area	18.3 ± 2.6	19.9 ± 1.9	14.9 ± 2.0	20.5 ± 3.0	23.3 ± 3.2	18.8 ± 3.8
Nuclear FF	0.742 ± 0.043	0.609 ± 0.051	0.667 ± 0.051	0.664 ± 0.027	0.502 ± 0.082	0.836 ± 0.041
SD nuclear FF	0.160 ± 0.016	0.163 ± 0.014	0.150 ± 0.010	0.147 ± 0.004	0.203 ± 0.019	0.105 ± 0.024
CV nuclear FF	21.8 ± 3.1	27.1 ± 3.7	22.7 ± 2.7	22.2 ± 1.3	42.2 ± 4.9	12.8 ± 3.4

*RL indicates reactive lymphocytosis; CB, cord blood; SS, Sézary syndrome; B-CLL, chronic lymphocytic leukemia of B-cell type; CV, coefficient of variation; and FF, form factor.

Table 2.—Statistical Comparison of Mean Ultrastructural Morphometric Nuclear Parameters Between Nonneoplastic and Neoplastic Lymphocyte Groups*

Parameter	N vs RL (A)	N vs SS	CB vs SS	RL (C) vs SS	RL (A) vs SS	N vs B-CLL	RL (A) vs B-CLL	B-CLL vs SS
Nuclear perimeter, μm	<.01†	<.001†	<.05†	<.05†	<.02†	>.9	<.05†	<.001†
SD nuclear perimeter, μm	>.1	<.001†	<.01†	<.001†	<.01†	<.01†	<.02†	<.001†
CV nuclear perimeter	>.4	<.001†	<.01†	<.001†	<.01†	<.001†	<.01†	<.001†
Nuclear area, μm^2	>.2	>.9	>.1	<.05†	>.5	<.01†	>.05	<.05†
SD nuclear area, μm^2	>.05	<.01†	>.4	<.02†	>.4	<.05†	>.5	>.5
CV nuclear area	>.1	<.01†	<.02†	<.001†	>.1	>.5	>.4	<.05†
Nuclear FF	<.001†	<.001†	<.01†	<.001†	<.001†	<.001†	<.001†	<.001†
SD nuclear FF	<.01†	<.001†	<.001†	<.001†	<.001†	<.001†	<.001†	<.001†
CV nuclear FF	>.5	<.001†	<.001†	<.001†	<.001†	<.001†	<.001†	<.001†

*N indicates normal; RL (A), reactive lymphocytosis (adults); SS, Sézary syndrome; CB, cord blood; RL (C), reactive lymphocytosis (children); B-CLL, chronic lymphocytic leukemia of B-cell type; CV, coefficient of variation; and FF, form factor. Data are given as *P* values throughout.

†Statistically significant.

3C) with Figs 1H, 2F, and 2G, and a granular lymphocyte from an expanded population of NK-like cells (Fig 3I) with Figs 1I and 2I.

The patients with viral-induced lymphocytosis revealed a greater number of lymphocytic nuclei that had lobated nuclei (Figs 2E, 2F, and 2G), stalked nuclear lobes ("drumsticks" or nucleotesimals), and fingerlike projections (Fig 2H) compared with that in normal subjects. An assessment of the proportion of nuclei with irregular profiles in different populations of nonleukemic subjects is most subjective. Morphometric methods were, therefore, utilized to provide an objective database for use in the diagnosis of leukemias, with an emphasis on SS.

Ultrastructural Morphometric Findings

Computerized Planimetric Methods Define Distinct Lymphoid Populations.—The individual nuclear perimeter (P), area (A) and FF values for the 87 nonleukemic and leukemic cases used were determined. The nonleukemic cases consisted of 20 normal subjects (10 female and 10 male), 15 neonates, 10 children with viral-induced lymphocytosis, and 5 adults with infectious mononucleosis. The leukemic cases consisted of 8 patients with SS, 10 patients with B-cell CLL, 15 patients with a variety of lymphocytic leukemias, some of which can be confused with SS (eg, T-cell CLL and LPL), and 4 patients with NK LPD.

Mean group values (Table 1) were then determined to compare statistically the patients who had SS with other groups with which they could be confused morphologically (Table 2). It was determined that patients with SS could be distinguished from normal subjects and adults with RL, on the basis of eight and six distinct morphometric nuclear features and mathematically derived parameters, respectively (Table 2). From a biologic standpoint, the lymphocytes from patients with SS could also be distinguished from cord blood lymphocytes (Table 2), which normally have more irregular nuclear profiles than adult lymphocytes.[49]

From a subjective standpoint, adults with infectious mononucleosis had a

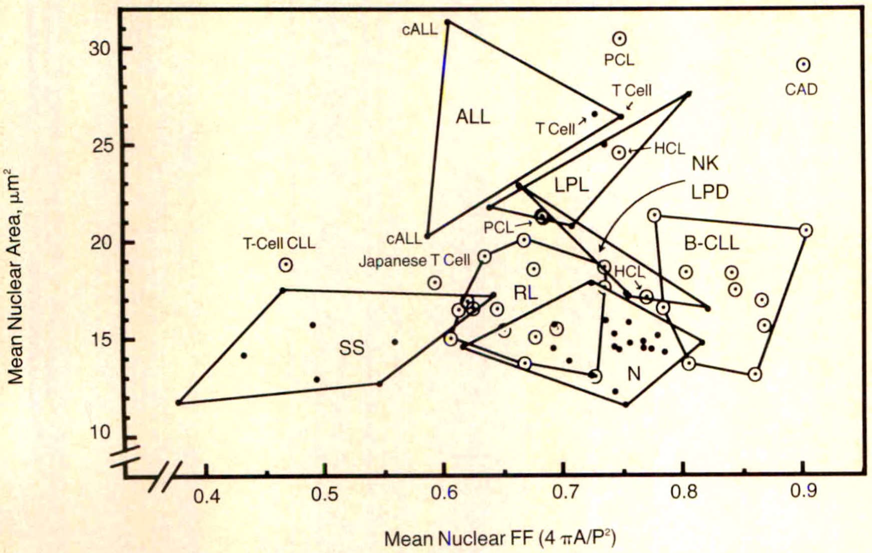

Fig 4.—Morphometric domains of normal subjects and patients with reactive lymphocytosis, leukemias, and natural killer cell lymphoproliferative disorders (NK LPDs). cALL indicates common acute lymphocytic leukemia; PCL, plasma cell leukemia; CAD, cold agglutinin disease; LPL, leukemic phase of lymphoma; HCL, hairy-cell leukemia; CLL, chronic lymphocytic leukemia; Japanese T Cell, Japanese T-cell leukemia; RL, reactive lymphocytosis; B-CLL, B-cell chronic lymphocytic leukemia; SS, Sézary syndrome; N, normal; FF, form factor; A, area, and P, perimeter.

greater number of lymphocytes with irregular nuclear contours compared with that in normal subjects. From an objective morphometric and statistical standpoint, these two populations of lymphocytes could indeed be distinguished on the basis of the mean nuclear perimeter (P), mean nuclear FF, and mean SD of the nuclear FF (Table 2). The reactive lymphocytes had a greater nuclear perimeter (P), a greater irregularity of nuclear profile, and a less inherent variability in nuclear shape than lymphocytes from normal subjects, indicating, perhaps, an expansion of distinct lymphocyte subpopulations in response to specific viral infections in reactive lymphocytes.

Multiparameter Analysis and the Establishment of Distinct Morphometric Domains.—It can be seen from the large number of statistically significant differences shown in Table 2 that leukemic populations may be more easily compared and visualized in a bivariate graphic display by using the combination of two different discriminatory nuclear parameters, ie, size and shape (Fig 4). Distinct morphometric domains are represented by polygons that define the morphometric boundaries of each group of leukemic patients and non-leukemic control subjects. The group with SS is far removed from the B-cell CLL group and closer to the normal subjects, but with no overlap. Since the eight patients with SS selected for this study had unequivocal circulating Sézary cells, as judged by an experienced hematopathologist (L.G.), it is possible that an overlap may exist between the morphometric domains of normal subjects and patients with SS who are less leukemic at initial presentation. A small amount of overlap is present between the group with SS and the patients with RL. The morphometric domain of the patients with SS is, however, far removed from the patients with LPL, with which it can be confused, morphologically. The patients with T-cell CLL and Japanese T-cell leukemia have larger nuclei than patients with SS and lie just outside the morphometric domain of the latter. More cases are needed, however, to assess the significance of this finding.

The patients with an NK LPD occupy a morphometric domain distinct from that of normal subjects and far removed from patients with chronic leukemias of T-cell origin, such as SS, T-cell CLL, and Japanese T-cell leukemia (Fig 4). Patients with acute lymphocytic leukemia, which can be easily distinguished from chronic neoplastic LPDs, were included for biologic interest only, and can be seen also to occupy a distinct morphometric domain by using bivariate graphic display techniques (Fig 4).

Evaluation of Sharply Angled NIs Using the Histogram Method.—The FF is sensitive to any deviation from a circle. Ellipses with smooth contours and nuclei with sharply angled NIs may have similar FF values. All cases were therefore evaluated by using a simple histogram technique that quantitates sharply angled NIs, and this technique was previously reported by our laboratory to be most helpful in distinguishing cases of MF from reactive lymphoid infiltrates of skin.[27,30] Figure 5 compares the mean histogram values ±2 SDs from normal subjects with other selected lymphoid populations. Figure 6 compares the range of histogram values from patients with SS with the values in the same selected lymphoid populations shown in Fig 5.

Although mean FF values served to distinguish patients with viral-induced lymphocytosis from normal subjects, the two groups were virtually indistinguishable by using the histogram method (Fig 5, top left). This indicates that although the reactive lymphocytes had a greater proportion of lobated nuclei (Figs 2E, 2F, and 2G) compared with that in normal subjects, the nuclear profiles did not have an unusually high number of sharply angled NIs that are commonly seen in malignant Sézary nuclei. The patients with SS, therefore, displayed a characteristic histogram pattern that served to distinguish the nuclei of these neoplastic cells from those of normal subjects (Fig 5, bottom left; Fig 6, top left), patients with RL (Fig 6, top right), NK LPDs (Fig 6, center left), B-cell CLL (Fig 6, center right), and LPL (Fig 6, bottom left). The histogram pattern of the two chronic T-cell leukemias (T-cell CLL and Japanese T-cell leukemia) overlapped, however, with that of the patients with SS.

COMMENT

The nucleus, in particular, has received much attention from morphometrists[50-56] because of the well-known relationship of nuclear structure to tumor aggressiveness,[57] the state of cell differentiation,[28,40,49,58-60] and the stage of the cell cycle.[61,62] In the present study, ultra-

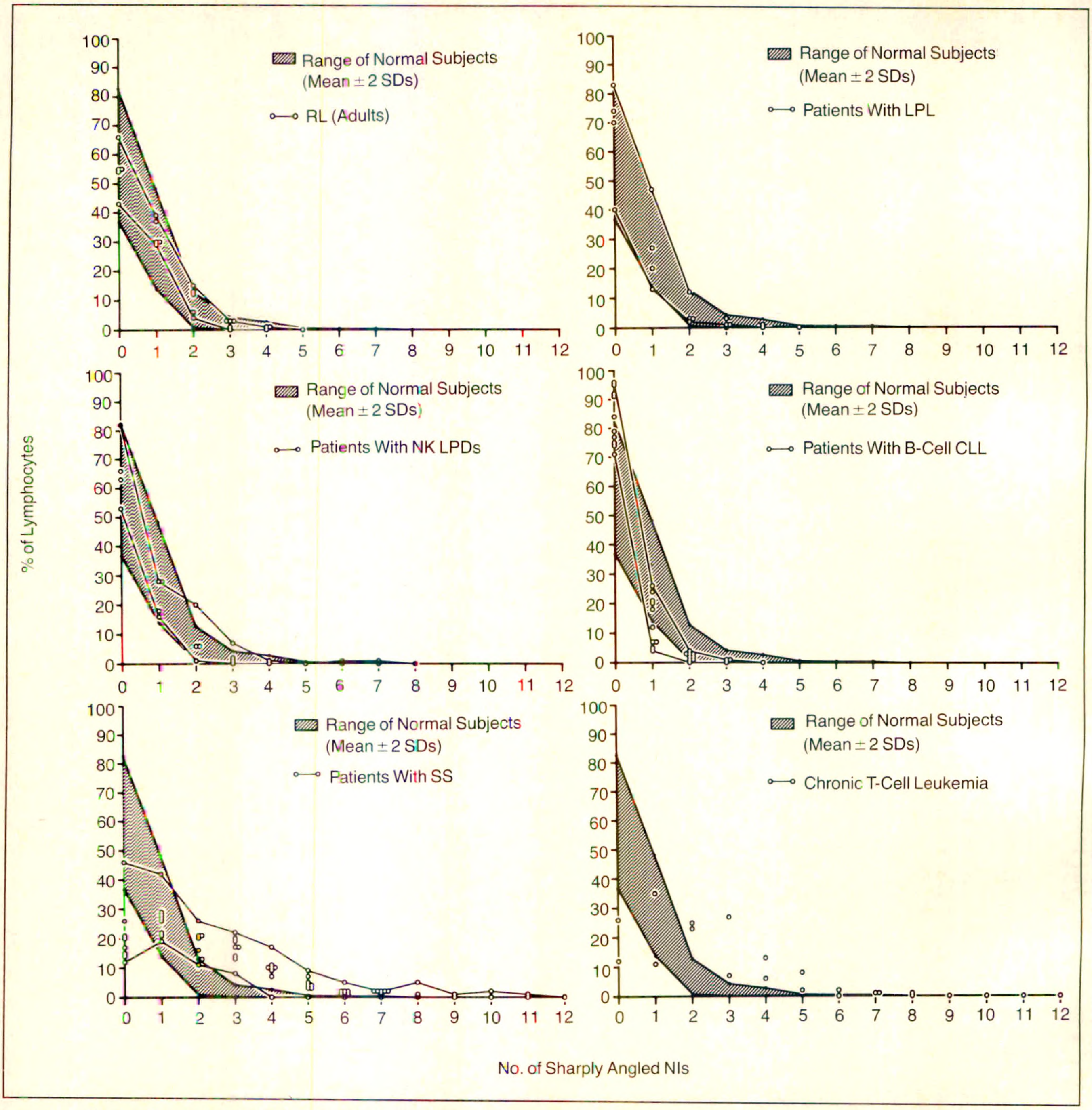

Fig 5.—Comparison of histogram values (percentage of lymphocytes having 0 to 12 sharply angled nuclear invaginations (NIs) from normal subjects with selected leukemic and nonleukemic lymphoid populations of cells. Sharply angled NIs are defined in the "Patients and Methods" section. The shaded area in each panel represents the normal range of values that was obtained by calculating the mean percentage of nuclei having 0 to 12 sharply angled NIs ± 2 SDs. The actual range of values for each of the other clinical groups is then compared with the normal range in each of the panels. Range boundaries are not drawn for the group with chronic T-cell leukemia for statistical reasons, as there were only two cases available for evaluation. RL indicates reactive lymphocytosis; LPL, leukemic phase of lymphoma; NK LPDs, natural killer cell lymphoproliferative disorders; CLL, chronic lymphocytic leukemia; and SS, Sézary syndrome.

structural morphometric techniques were applied to the nuclei of circulating lymphoid cells from normal subjects, patients with RL, and patients with different types of chronic lymphoid leukemias, to provide a database for examining patients with SS. At the light microscopic level, the appearance of the chronic lymphoid leukemias that consist of small to medium cells with abundant heterochromatin can present certain diagnostic difficulties,[63] and multiparameter approaches are recommended.[64-66] These chronic LPDs and allied disorders include SS, B-cell CLL, T-cell

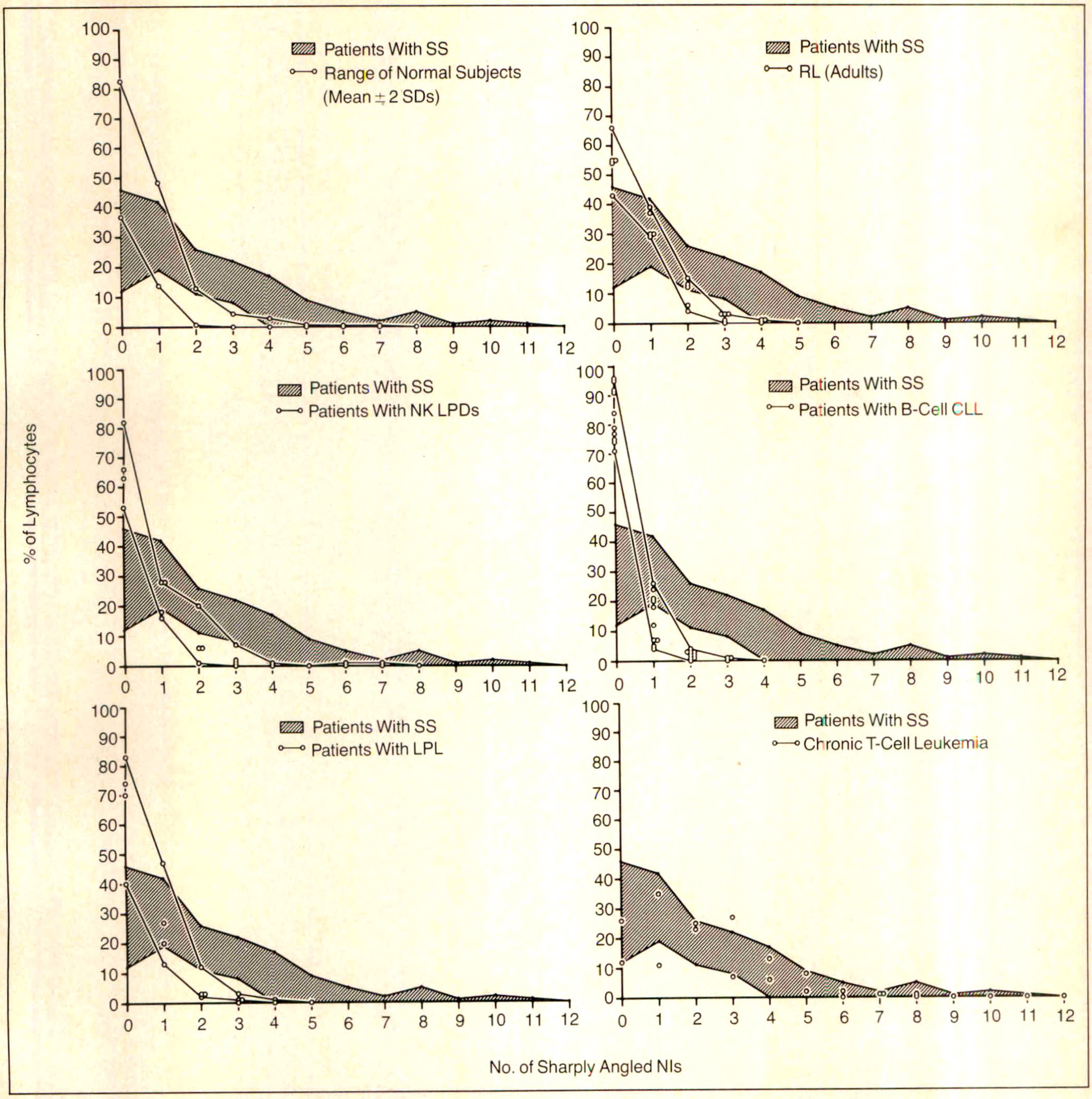

Fig 6.—Comparison of histogram values (percentage of lymphocytes having 0 to 12 sharply angled nuclear invaginations (NIs) from patients with Sézary syndrome (SS) with the same selected lymphoid populations shown in Fig 5. The shaded area in each panel represents the actual range of values obtained from the eight patients in the group with SS. RL indicates reactive lymphocytosis; NK LPDs, natural killer cell lymphoproliferative disorders; CLL, chronic lymphocytic leukemia; and LPL, leukemic phase of lymphoma.

CLL, leukemic phase of well-differentiated lymphocytic lymphoma, hairy-cell leukemia, adult T-cell leukemia (Japanese or knobby variant), NK LPDs [Tγ-LPD, LGL (large granular lymphocyte) T-cell CLL], and plasma cell leukemia. The morphological difficulties are compounded by the fact that the neoplastic cells from patients with SS can have tartrate-resistant acid phosphatase activity,[67] a characteristic of the hairy cells from patients with hairy-cell leukemia.[68] In addition, the leukemic cells from patients with adult T-cell leukemia can form Pautrier's microabscesses within the epidermis,[69,70] thereby mimicking MF.

It has been reported in the literature that transmission electron microscopy is the method of choice for documenting[1,3,7] and quantitating[41,71-74] the distinctive convolutions that characterize the Sézary cell.[10] Unfortunately, cerebriform[75] or Sézary-like[13] cells are increased in number in the peripheral circulation of patients who have rheumatic disease,[75] sepsis,[13] and viral-induced lymphocytosis (present study). Statistical analyses of populations of cells are, however, more revealing about the neoplastic vs reactive nature of the cells. In the present study, it was determined that ultrastructural morphometric techniques, when applied to populations of mature-appearing lymphoid cells, revealed the underlying "geometric monoclonality"[52] of the malignant cell proliferations. It was determined that patients with SS could be distinguished from normal subjects and patients with RL and other types of lymphoid leukemias by using means, SDs, and CVs of distinct nuclear features. Obviously, if a patient's mean value falls within an overlap zone between groups or within 2 SDs of the "outlier" points of a given morphometric domain, a definitive diagnosis would not be possible on an individual basis, by using the two variables being considered.

From a practical standpoint, the simple histogram method[27,30] that quantitates the number of sharply angled NIs is the method of choice over computerized planimetric techniques for the evaluation of blood specimens from patients whose conditions were previously diagnosed as MF. In everyday practice, we routinely use the histogram method to diagnose patients with suspected SS. We have found this scoring method to be more sensitive than mean FF values determined from computerized planimetry and more sensitive than light microscopy (C.M.P, L.G., and T. M. Grogan, MD, unpublished data, 1990). The reason for this is a reflection of the fact that FF does not discriminate between an ellipse with a smooth contour and a convoluted cell with a rough contour if the area and perimeter values are identical between the two images,[39] whereas the human eye does. The diagnostically useful histogram method is somewhat analogous, although not identical, to the diagnostically useful nuclear lobe count in neutrophils. By using the more tedious bivariate graphic display technique, the morphometric domains of the group with RL and the group with SS overlapped slightly, whereas the histogram patterns were most distinct. The histogram technique was previously used to distinguish patients with MF from those with chronic dermatitis.[27,30] In fact, the histogram technique was later confirmed by Willemze et al[38] to produce no false positives in their series of cases.

It has recently been suggested that multiple descriptors be extracted from the nucleus to have a practical application in anatomic pathology.[76] The present study, by using bivariate displays of nuclear size and shape measurements as x,y coordinates, points out the value of such a multiparameter approach. Seven populations of reactive and malignant lymphoid cells were shown to occupy distinct morphometric domains. It is noteworthy that the morphometric domain of the patients with LPL were clearly graphically separated from those of the reactive and SS groups, in keeping with the larger nuclear size previously reported by other investigators for LPL.[77] In addition, the two cases of T-cell CLL and Japanese T-cell leukemia that were indistinguishable from patients with SS by using the histogram method fell outside the morphometric domain of patients with SS on the basis of nuclear size alone. Computerized morphometric techniques may, therefore, be of greater value when the range of possible diagnoses is large. Previous studies from our laboratory, on other types of neoplasms, point out the biologic[48,78] and diagnostic[45-48,78,79] relevance to multiparameter analysis. Interesting cell organelle morphometric relationships are sure to emerge among the leukemias, as they have for the solid tumors.[45,48]

The future applications of ultrastructural morphometry to diagnosis and prognosis, coupled with quantitative correlations of nuclear features with indicators of biologic aggressiveness, such as proliferation antigens and ploidy analysis, should be most rewarding.

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Papillary Cystadenoma of the Epididymis

A Report of Three Cases With Lectin Histochemistry

Peter J. Kragel, MD; Joseph Pestaner; William D. Travis, MD; W. Marston Linehan, MD; Michele R. Filling-Katz, MD

• Papillary cystadenoma of the epididymis is an uncommon benign tumor associated with von Hippel-Lindau disease. Since metastatic renal cell carcinoma may be histologically similar to papillary cystadenoma, and both are associated with von Hippel-Lindau disease, differentiation between these two entities may be difficult. We performed lectin histochemistry studies on three papillary cystadenomas and compared the results with the staining observed in epididymal ducts, epididymal efferent ductules, and three renal cell carcinomas. Common positive staining was observed following incubation with soybean agglutinin in epididymal ducts and two of the three papillary cystadenomas, while the three renal cell carcinomas did not stain. When epididymal tumors histologically consistent with papillary cystadenoma fail to react with soybean agglutinin, thorough clinical evaluation for an occult renal cell carcinoma should be performed.

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Papillary cystadenoma of the epididymis is an uncommon benign tumor considered to be a hamartoma rather than a true neoplasm, which is often associated with von Hippel-Lindau disease.[1] Histogenesis from epididymal ducts has been suggested,[1-5] but it is disputed by some authors.[6-10] In patients with von Hippel-Lindau disease, differentiation of papillary cystadenoma from metastatic renal cell carcinoma may present a diagnostic challenge, since the incidence of both is increased in von Hippel-Lindau disease, and the histologic features of both may be similar. We performed histochemical stains with a panel of lectins on three cases of papillary cystadenoma and three cases of renal cell carcinoma. Our results support origin of papillary cystadenoma from epididymal ducts and show different patterns of lectin staining for papillary cystadenoma and renal cell carcinoma (Table).

REPORT OF CASES

CASE 1.—A 65-year-old man with stage IV nodular mixed cell lymphoma and transitional cell carcinoma of the urinary bladder presented with increasing nausea and vomiting and hepatic and renal failure. His lymphoma was diagnosed at age 55 years. He was treated with CHOP (a drug regimen consisting of cyclophosphamide, doxorubicin, prednisone, and vincristine sulfate) and C-MOPP (a drug regimen consisting of cytoxan, mechlorethamine hydrochloride, vincristine, procarbazine hydrochloride, and prednisone) and achieved complete remission He was further treated with low-dose radiation therapy; he received 24 Gy to the mantle zone, 22.4 Gy to the upper abdomen, and 6 Gy to the pelvis. His lymphoma recurred at age 62, at which time he presented with hematuria, scrotal edema, and inguinal adenopathy. Results of a bone marrow biopsy were positive for lymphoma. Follow-up cystoscopy and results of biopsy at age 63 showed a grade 2 papillary transitional cell carcinoma without invasion. A liver biopsy specimen obtained during this hospital admission showed metastatic carcinoma. The patient died following a hypotensive episode; permission for autopsy was obtained. An incidental finding at autopsy was a 1.0-cm mass in the head of the epididymis.

CASE 2.—A 25-year-old man with von Hippel-Lindau disease was admitted to the hospital for evaluation of a pheochromocytoma with complaints of increased sweating, fatigability, and constipation. He was first diagnosed as having von Hippel-Lindau disease at age 11 years when the disease manifested itself with retinal angiomatosis that lead to blindness in both eyes by the time he was 22 years old. The patient had a cerebellar hemangioblastoma resected at age 20 years.

Abdominal ultrasound examination showed a 2.5-cm right adrenal mass and a cystic mass in the head of the pancreas. Testicular ultrasound examination demonstrated a cystic mass in the right side of the

Lectin Staining of Papillary Cystadenoma, Renal Cell Carcinoma, Epididymal Ducts, and Epididymal Efferent Ductules*

	Lectins						
	WGA	RCA	DBA	UEA I	ConA	SBA	PNA
PCE 1	+++	++	wf	−	+	++	++
PCE 2	++	++	−	−	+++	++	+++
PCE 3	++	+++	−	−	++	−	+
RCC 1	+++	+++	−	−	++	−	+
RCC 2	+++	+++	−	−	++	−	+
RCC 3	+++	+++	+	−	++	−	+
EP DUC	++	++	−	−	++	++	++
EFF DU	++	++	+	++	++	−	++

*PCE indicates papillary cystadenoma of the epididymis; RCC, renal cell carcinoma; EP DUC, epididymal ducts; EFF DU, epididymal efferent ductules; triple plus sign, strong positive staining; double plus sign, moderate positive staining; plus sign, weak positive staining; wf, weak focal positive staining; minus sign, no positive staining; WGA, wheat germ agglutinin; PNA, peanut agglutinin; SBA, soybean agglutinin; ConA, concanavalin A; DBA, *Dolichos biflorus* agglutinin; UEA I, *Ulex europaeus* agglutinin I; and RCA, *Ricinus communis* agglutinin.

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epididymis. Laboratory evaluation showed the following values: plasma norepinephrine, 7.45 pmol/L; and plasma epinephrine, 284 pmol/L. Physical examination was remarkable for a blood pressure of 164/110 mm Hg, a pulse rate of 116 beats per minute, and a grade 2/6 systolic ejection murmur at the left sternal border and apex. Right adrenalectomy, with subsequent pathologic specimens showing a 2.0-cm pheochromocytoma, and right testicular surgical exploration were performed.

CASE 3.—A 60-year-old man with von Hippel-Lindau disease, the father of patient 2, was noted on physical examination to have a cystic mass in the left part of the scrotum. Ultrasonography disclosed a cystic mass in the left side of the epididymis and a nonhomogeneous area within the testis that was suspicious for a malignant neoplasm. He was first diagnosed as having von Hippel-Lindau disease at 34 years when craniotomy for a posterior fossa hemangioblastoma was performed. A pancreatic islet cell tumor was resected at age 53 years.

Findings from physical examination were remarkable for a 3.0-cm spherical cyst in the left epididymal area. Findings from laboratory evaluation were noncontributory. Left inguinal surgical exploration followed by left orchiectomy were performed.

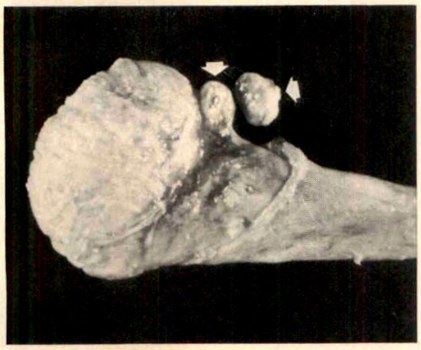


Fig 1.—Macroscopically, the orchiectomy specimen from case 3 shows a well-circumscribed, 2.0-cm yellow to tan epididymal mass (arrow) with both solid and cystic areas.

MATERIALS AND METHODS

Tissues were fixed in 10% formaldehyde solution, processed routinely, and embedded in paraffin. Microtomy sections of the three cases of papillary cystadenoma of the epididymis and three cases of renal cell carcinoma from the files of the Laboratory of Pathology, the National Cancer Institute (Bethesda, Md), were stained with hematoxylin-eosin, periodic acid–Schiff (PAS) with and without diastase digestion, and alcian blue histochemical stains. Additional sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked with 2% hydrogen peroxide for 5 minutes. The slides were next flooded with undiluted non–immune horse serum samples for 10 minutes, then rinsed in TRIS buffer for 10 minutes. Biotinylated wheat germ agglutinin, peanut agglutinin, soybean agglutinin, concanavalin A, *Dolichos biflorus* agglutinin, and *Ulex europaeus* agglutinin I (Vector Laboratories, Burlingame, Calif) were reconstituted in 0.5 mL of distilled water. *Ricinus communis* agglutinin I (Vector) was provided in solution according to product specifications. All lectins were diluted 1:100 with TRIS buffer; tissue sections were flooded with a biotinylated lectin and incubated for 30 minutes. Negative controls were prepared by incubating with TRIS buffer rather than the biotinylated lectin. Slides were then washed three times in TRIS buffer and incubated with avidin-biotin complex peroxidase (Vector) for 60 minutes. Sections were next washed in TRIS buffer for 10 minutes and incubated with diaminobenzidine in 0.01% hydrogen peroxide for 10 minutes. Sections were washed in tap water for 5 minutes, counterstained with hematoxylin, dehydrated, and coverslipped.

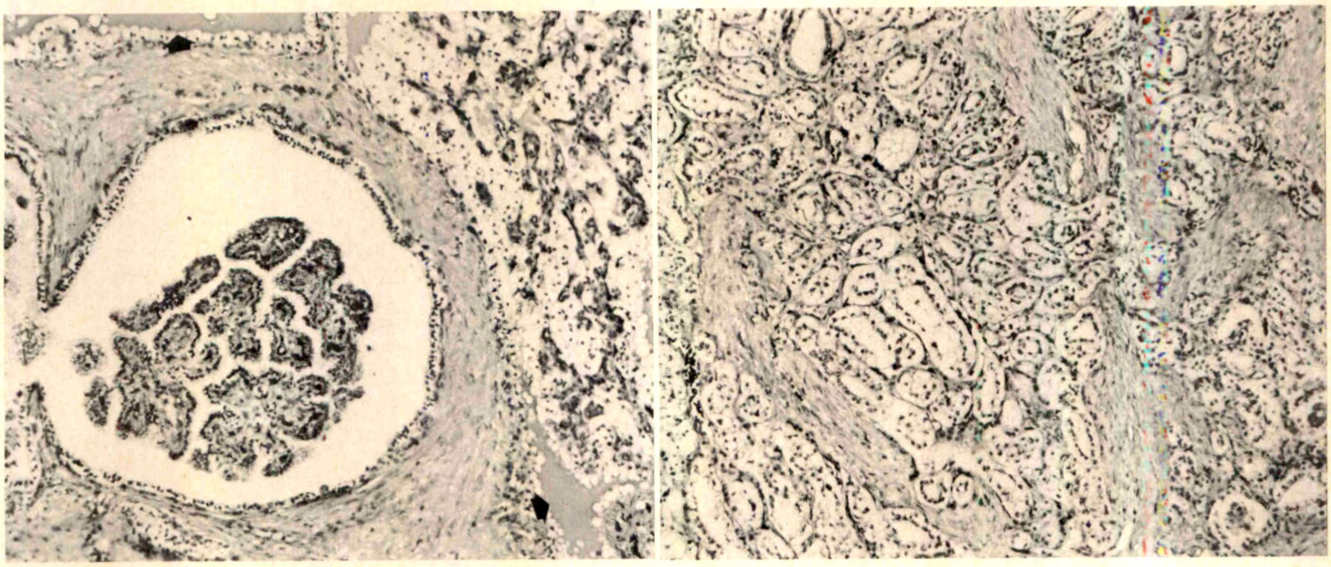
RESULTS

Macroscopic and Histologic Findings

Macroscopically, the papillary cystadenomas were yellow to tan, well circumscribed, and composed of solid areas (case 1) or both solid and cystic areas (cases 2 and 3 [Fig 1]). On pathologic gross examination, the lesions measured 2.0 cm (case 3), 1.0 cm (case 1), and 0.7 cm (case 2) in greatest dimension.

Microscopically, the tumors were composed of tubular and papillary arrangements of monomorphic cuboidal and columnar cells with optically clear cytoplasm and distinct cell borders. Some cyst spaces were filled with a colloidlike material. A dense fibrous stroma was also present (Fig 2). The cell cytoplasm contained coarse granular

Fig 2.—Microscopically, both papillary (left) and tubular arrangements (right) of monomorphic cells with clear cytoplasm and colloidlike material in cyst spaces (left, arrows) are present (left, case 3, hematoxylin-eosin, ×100; right, case 1, hematoxylin-eosin, ×100).



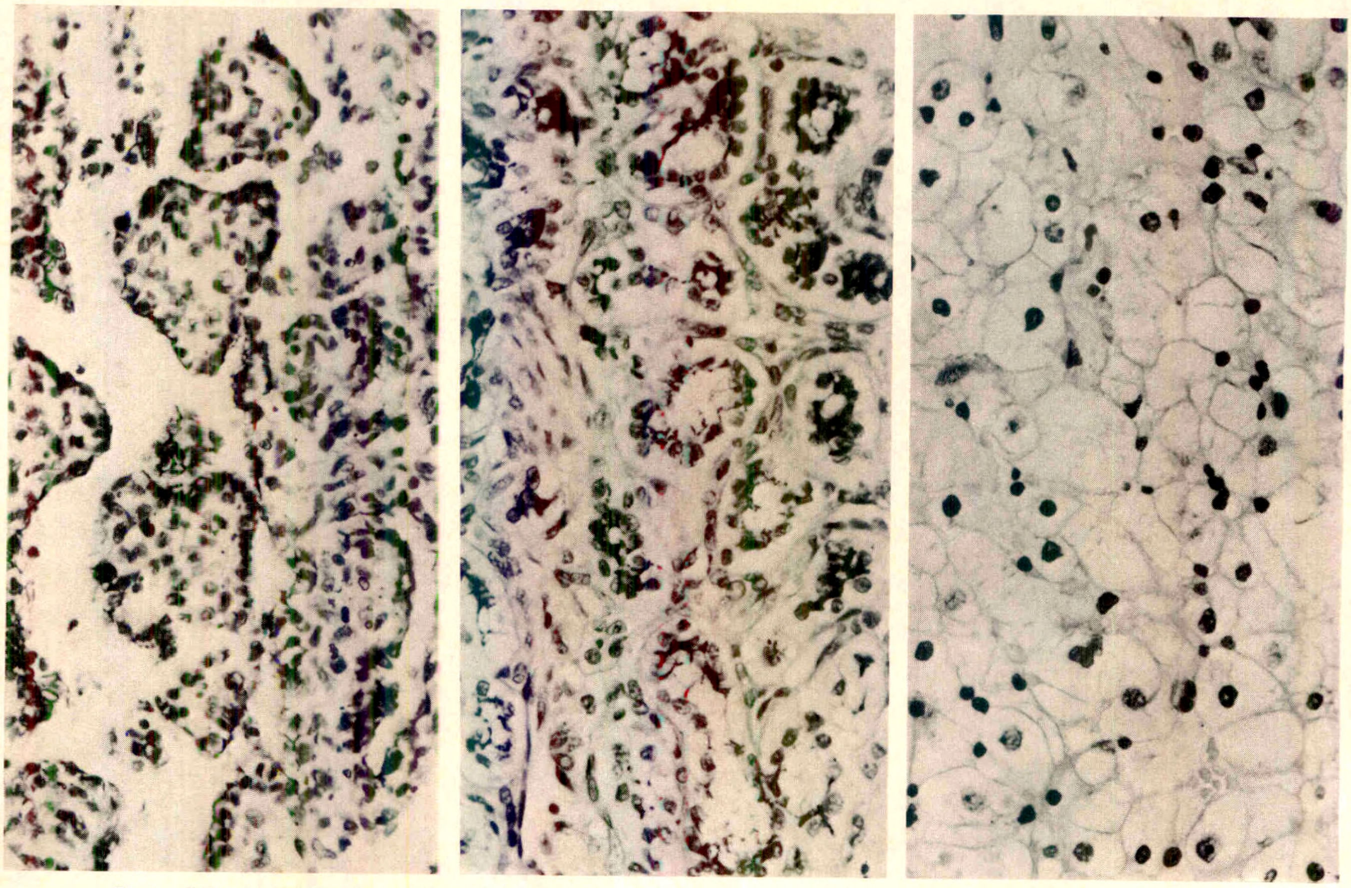
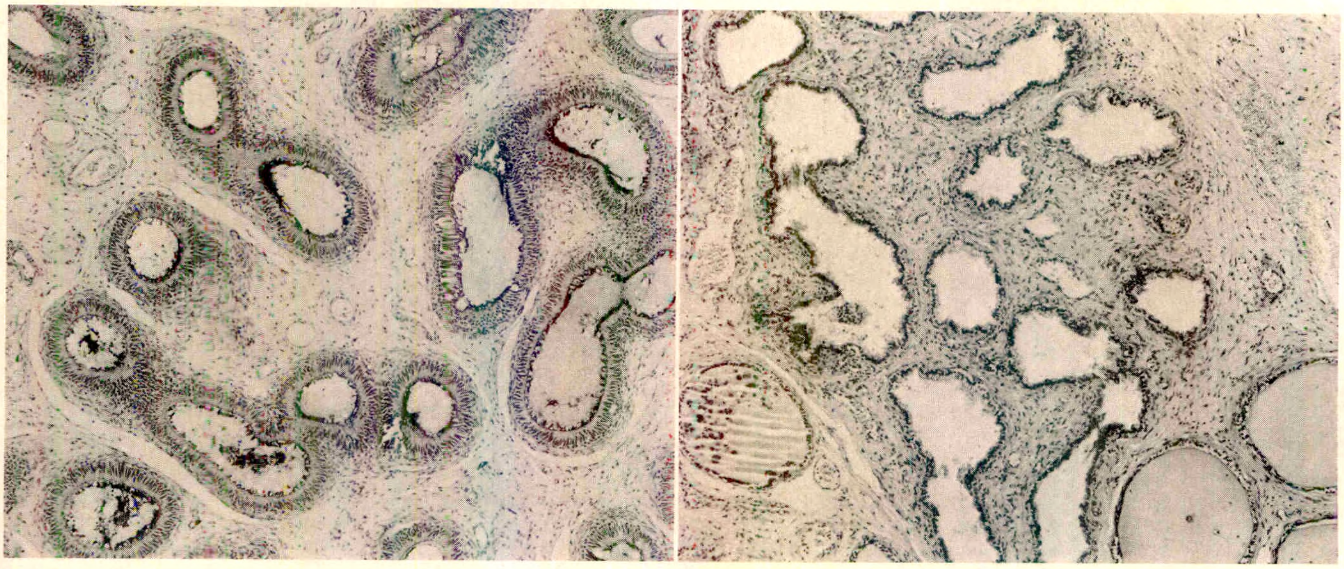


Fig 3.—Following incubation with biotinylated soybean agglutinin, positive staining is seen in two papillary cystadenomas (left, case 2; center, case 1) but not in renal cell carcinoma (right) (avidin-biotin complex–peroxidase with hematoxylin counterstain, ×250).

Fig 4.—Following incubation with biotinylated soybean agglutinin, positive staining is seen in epididymal ducts (left) but not in epididymal efferent ductules (right) (avidin-biotin complex–peroxidase with hematoxylin counterstain, ×100).



PAS-positive, diastase-sensitive material. Alcian blue stains showed focal, weak cytoplasmic positivity only in case 1. The colloidlike material in cyst spaces was PAS positive, diastase resistant. Epididymal ducts and efferent ductules were present adjacent to the papillary cystadenomas in cases 1 and 3.

Lectin Histochemical Findings

Lectin staining results are summarized in the Table. Wheat germ agglutinin, *Ricinus communis* agglutinin I, concanavalin A, and peanut agglutinin reacted with both papillary cystadenomas and renal cell carcinomas. Staining was present along the cell membranes and tubular lumina in papillary cystadenoma and along cell membranes in renal cell carcinoma. Epididymal ducts and efferent ductules also showed positive staining following incubation with these lectins. *Dolichos biflorus* agglutinin reacted with one case of papillary cystadenoma and one case of renal cell carcinoma. Staining was weak and focal. *Dolichos biflorus* agglutinin reacted with efferent ductules but not epididymal ducts. *Ulex europaeus* agglutinin did not react with either papillary cystadenomas or renal cell carcinomas. *Ulex europaeus* agglutinin did react with endothelial cells and efferent ductules but not with epididymal ducts. Soybean agglutinin reacted with two cases of papillary cystadenoma. Staining was observed along cell membranes and tubular lumina (Fig 3). Soybean agglutinin also reacted with epididymal ducts but not with efferent ductules (Fig 4). No cases of renal cell carcinoma reacted with soybean agglutinin.

COMMENT

The origin of epididymal papillary cystadenoma is still the subject of debate. Various authors have proposed epididymal ducts,[1-5] efferent ductules,[6-8] müllerian remnants,[9,10] mesonephric rests,[3,4] or spermatoceles[1,10] as probable sites of origin. An ultrastructural study demonstrating irregular microvilli is consistent with epididymal duct origin.[5] To our knowledge, lectin histochemical findings have not been reported prior to this study.

We performed lectin histochemical staining of three cases of papillary cystadenoma and compared the results with the staining observed in epididymal ducts, efferent ductules, and three cases of renal cell carcinoma. Significant differences in the positivity of these tissues were noted following incubation with soybean agglutinin, *D biflorus* agglutinin, and *U europaeus* agglutinin I; in particular, two of three papillary cystadenomas stained with soybean agglutinin while no renal cell carcinomas stained. The epididymal ducts were also positive following incubation with soybean agglutinin, while the efferent ductules were negative. *Dolichos biflorus* agglutinin and *U europaeus* agglutinin I stained efferent ductules but neither the epididymal ducts nor the papillary cystadenomas.

These lectin staining patterns demonstrate similarities in cell surface glycoproteins between papillary cystadenomas and epididymal ducts, suggesting common differentiation and probable origin of papillary cystadenoma from epididymal duct epithelium. Soybean agglutinin has been shown to preferentially react with *N*-acetylgalactosamine and α-galactosamine.[11] Positive staining following incubation with soybean agglutinin indicates the presence of these carbohydrates on the cell surface, perhaps as a component of an integral membrane proteoglycan.

Of the 2% to 4% of patients with renal cell carcinoma who have a solitary metastasis, approximately 4% will have a metastasis to the epididymis.[12] In these patients, differentiation of metastatic renal cell carcinoma from papillary cystadenoma may be clinically and histologically difficult. The histologic features of papillary cystadenoma, as characterized by the presence of tubules lined by cuboidal clear cells, proteinaceous material in cyst spaces, and papillary structures are distinctive; however, metastatic renal cell carcinoma as well as other metastatic papillary carcinomas may be histologically indistinguishable.[13]

Von Hippel-Lindau disease occurs in association with renal cell carcinoma in approximately 25% of patients,[14] and papillary cystadenoma of the epididymis has been reported to occur in association with von Hippel-Lindau disease in 38% of patients.[6] These associations serve to further complicate the differential diagnosis of epididymal papillary cystadenoma. Lectin histochemical staining may provide a means to differentiate epididymal papillary cystadenoma from renal cell carcinoma. In particular, soybean agglutinin staining was observed in papillary cystadenoma in two of three cases and was not observed in renal cell carcinoma.

In conclusion, the term "epididymal duct hamartoma" is recommended for these tumors. Their common association with von Hippel-Lindau disease and benign clinical behavior suggests that these tumors are hamartomas. Lectin histochemical stains show common differentiation of both papillary cystadenoma cells and epididymal duct cells. When epididymal tumors histologically consistent with epididymal duct hamartoma fail to react with soybean agglutinin, particularly in the setting of von Hippel-Lindau disease, thorough clinical evaluation to exclude an occult renal cell carcinoma should be performed.

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Lymphoblastic Crisis of Chronic Myelogenous Leukemia

Hand Mirror Variant

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Surbhi N. Desai, PhD; Harold Schumacher, MD

• We present, to our knowledge, the first extensively studied case of lymphoid L2 blast crisis of chronic myelogenous leukemia with a hand mirror cell (HMC) variant. Special stains revealed the leukemic cells to be terminal deoxynucleotidyl transferase positive by immunofluorescence and cytochemically positive for α-naphthyl acetate esterase and acid phosphatase (diffuse granular). Immunophenotyping identified the major leukemic cell population as B-cells that expressed CD10$^+$, CD19$^+$, and HLA-DR$^+$. It was not possible to separate the HMC and the non–HMC leukemic population by gating various cell populations, dual staining, cytochemistry, or by terminal deoxynucleotidyl transferase. Gene rearrangements were observed in both Ig heavy-chain alleles and one T-cell antigen receptor γ-subunit allele. The rearrangements occupied all of the cells, indicating that the HMC and non-HMC were of a common clonal origin. The patient had a mosaic karyotype, with 90% of the cells having t(9;22), t(8;14), and t(9;15) translocations, an additional chromosome 8, and deleted chromosomes 9 and 15. Antibodies to simian sarcoma–associated virus and baboon endogenous virus were isolated in the patient's peripheral blood plasma.

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Hand mirror cells (HMC) have been associated with normal hematopoietic elements,[1] acute leukemias,[2-5] Burkitt's lymphoma,[6] and the recovery phase of infectious mononucleosis.[7] One case has been described of their occurrence in a lymphocytic blast crisis of a chronic myelogenous leukemia, but detailed analysis was not performed.[8] We present the first case, to our knowledge, with an extensive workup of the lymphoid blast crisis, HMC variant, in chronic myelogenous leukemia.

REPORT OF A CASE

A 60-year-old Indian man was admitted to Loyola University Medical Center (Maywood, Ill) in February 1988, with a 4-day history of gingival bleeding, and a 3-week period of progressive weakness, weight loss of 13.5 kg, and night sweats without chills or fever. Chronic myelogenous leukemia had been diagnosed 1 year before the development of these symptoms and he had been treated with hydroxyurea.

On admission, he had a blood pressure of 118/80 mm Hg, heart rate of 96 beats per minute, temperature of 38.2°C, and a respiratory rate of 20 per minute. Physical examination revealed a palpable spleen tip, mild left facial droop with decreased pinprick sensation over the left cheek and left eyelid, and gingival bleeding. Results of laboratory studies on admission revealed the following: white blood cell count, 89 × 10^9/L, with 91% blast cells; hemoglobin, 137 g/L; and platelet count, 25 × 10^9/L.

A bone marrow aspiration and biopsy confirmed a lymphoid blast crisis with an HMC component of 32% and blast cells, 62% (Figure).

He was treated by leukapheresis, and then a 9-day course of cytarabine (cytosine arabinoside) at 2 g/m^2 and amsacrine (AMSA) at 100 mg/m^2 was begun. He went into remission within 1 month and did well until he had

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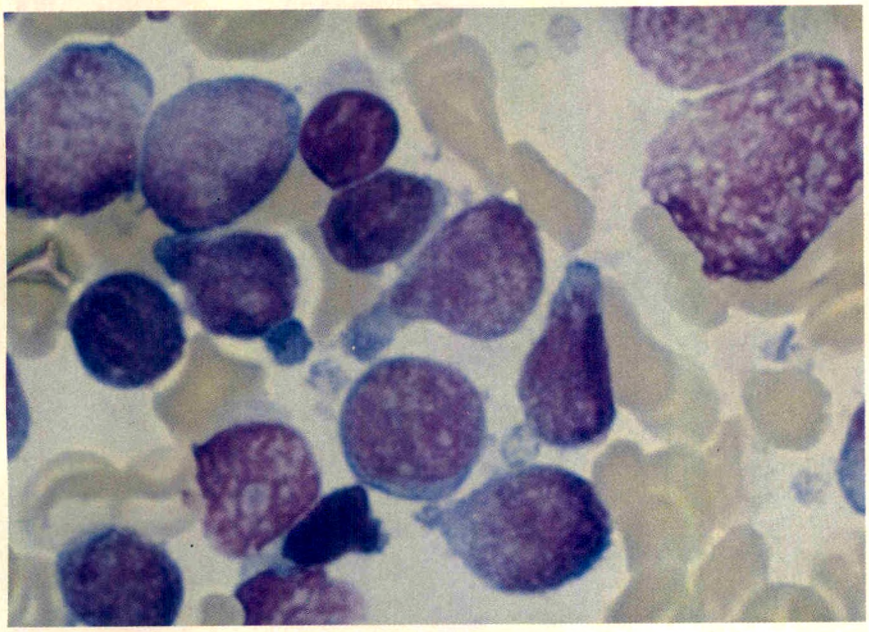

Bone marrow aspirate showing hand mirror cells (original magnification × 1000).

a relapse in October 1988, with 70% blast cells and 38% HMC.

He was treated with vincristine, prednisone, and L-asparaginase, and a second remission was induced.

METHODS

Peripheral Blood and Bone Marrow

Studies were performed at diagnosis, remission, and relapse. Peripheral blood smears and bone marrow aspirate smears were stained with May-Grünwald/Giemsa, and the bone marrow biopsy specimen and clot were fixed in B-5 and examined by routine hematoxylin-eosin preparations.

Cytochemistry and Terminal Deoxynucleotidyl Transferase (TdT)

Cell suspensions from bone marrow were studied by cytochemical methods. The enzymes investigated were acid phosphatase, α-naphthyl acetate esterase, α-naphthyl butyrate esterase, chloroacetate esterase, periodic acid–Schiff reaction, Sudan black B, and myeloperoxidase.[9] Terminal deoxynucleotidyl transferase testing was performed by indirect immunofluorescence.[10]

Immunophenotyping

Surface marker analyses at diagnosis and relapse were performed using Ficoll-Hypaque separated blood and bone marrow cells. These cells were stained by direct or indirect techniques, using monoclonal antibodies directed to T-cells (CD5, CD8, CD3, and CD7, Becton Dickinson Immunocytometry Systems, Mountain View, Calif); B-cells (CD19, Coulter Immunology, Hialeah, Fla); kappa and lambda immunoglobulin light chains; IgM, IgD, IgG, and IgA heavy chains (Kallestad Laboratories Inc, Austin, Tex); myeloid cells (CD12, CD33, CD11, CD14, and My8, Coulter Immunology); CD10 (Coulter Immunology); and TdT antibodies (Molecular Genetic Resources, Tampa, Fla) were also used.

The cells were analyzed using a flow cytometer (FACScan, Becton Dickinson). One major cell population with light scatter characteristics of lymphocytes was observed and analyzed by one- and two-color fluorescence histograms and displayed on a logarithmic scale. The results were reported as percent of the total gated population.

Gene Rearrangement

High-molecular weight DNA was prepared from mononuclear cells isolated by Ficoll-Hypaque density centrifugation. Methods and probes used in Southern blot analyses to detect clonal immunoglobulin and T-cell antigen receptor gene rearrangements were performed as previously described.[11]

Cytogenetics

Cytogenetic analysis with trypsin Giemsa's banding technique was performed on bone marrow in the laboratory of Janet Rowley, MD, University of Chicago (Ill). The criteria adopted at the Fourth International Workshop on Chromosomes in Leukemia were used for evaluation.[12]

Western Blot Analysis

Western blot analysis was performed on the patient's peripheral blood plasma and blast cells at the time of blast crisis according to the method previously published.[13] The antibodies studied were those against the simian sarcoma–associated virus (SSAV) and baboon endogenous virus (BaEV). The SSAV and BaEV grown in human cell lines, and antibodies made in goat to these viruses were obtained from Microbiological Associated, National Institutes of Health, Bethesda, Md. Control cells were normal lymphocytes obtained from healthy laboratory personnel and separated by Ficoll-Hypaque. One control was stimulated with phytohemagglutinin and the other was unstimulated.

RESULTS

Morphology

The bone marrow blast cells demonstrated L2 morphology. The HMC consisted of both blast cells and lymphocytes with more mature nuclei. The nucleus occupied the "mirror" portion of the cell, whereas the tail or uropod contained slightly basophilic cytoplasm. The bone marrow blast cells and HMC were α-naphthyl acetate esterase positive, α-naphthyl butyrate esterase negative, myeloperoxidase negative, Sudan black B negative, periodic acid–Schiff negative, combined esterase positive for the α-naphthyl acetate esterase portion and negative with chloroacetic esterase. The lymphoblasts and HMC showed a diffuse granular positivity on acid phosphatase, which was sensitive to tartrate.

Immunophenotyping

Immunophenotyping showed the malignant B-cells (32% HMC and 62% blast cells, combined) to express antigens CD19 (87%), CD10 (89%), HLA-DR (94%), and TdT (90%), with no expression of cytoplasmic or surface immunoglobulin heavy or light chains. Cells were specifically tested in two-color fluorescence to determine whether any T-cell antibodies reacted with the B-cell population. There were two separate populations (T-cell [10%] and B-cell [90%]) by immunophenotyping, however, it was not possible to separate out the HMC component as a separate entity. The 10% normal population by flow cytometry was a T-lymphocyte population that expressed 8% $CD3^+$, with 5% CD4 and 3% CD8 cells.

Gene Rearrangement

The vast majority of mononuclear cells in the February 1988 bone marrow aspirate appeared to be clonal with both of their heavy-chain alleles rearranged. The genes for the kappa light chain were in germline configuration. The genes for the β-subunit were not found to be clonally rearranged.

The γ-subunit loci exhibited one allele in germline configuration and the other clonally rearranged to the second joining region with a concomitant deletion of the first joining region. No clonal rearrangements were detected in the April 1988 remission peripheral blood specimen. On relapse in October 1988, the same pattern of clonal rearrangements, both in type and degree, were detected as had been observed in February 1988.

Cytogenetics

Chromosome studies showed a random loss of chromosomes due to cell breakage, and a mosiac karyotype: 45,XY(5%) / 46,XY,t (9;22) (q34;q11) (5%) / 46,XY, =8,-9,-15,t (8;14) (q11; q32),t (9;22), +der (9)t (9;15) (p13;q11) (90%).

Western Blot Analysis

Both the BaEV and SSAV p30 antigens were recognized by the anti-IgM antibodies from the patient's peripheral blood plasma. The reaction was strongly positive against the SSAV p30 antigen. The normal controls sometimes showed a faint cross-reaction with the p30 antigen from SSAV. The surface protein gp70 was not recognized by the IgM antibody in the SSAV Western blot, but was faintly positive for the BaEV. The IgM antibody was absent in the normal controls.

COMMENT

Blast crises in chronic myelogenous leukemia are usually of myelocytic, monocytic, or combined origin. A smaller portion of patients develop a lymphoid blast crisis. There is one brief report in the literature about the presence of HMC in a lymphoid blast crisis in chronic myelogenous leukemia that identified the blast cells as myeloperoxidase negative and TdT positive.[8] The significance of lymphoid HMC has been debated in the past. Hand mirror cells in acute lymphoblastic leukemia have been identified as early as B-cell lineage, although cases of early T-cell, B-cell, and myeloid lineages have been seen.[1,3,6]

Dual labeling in our patient identified the T-cells and B-cells as two separate cell populations, and the HMC were of the B-cell lineage. Our patient is unique in that he had a single clone of cells with both Ig heavy-chain rearrangements and a rearrangement at the γ-submit locus of the T-cell antigen receptor. B-cells may have a T-cell antigen receptor γ rearrangement that has limited significance as a marker of clonality and lineage.[14] The relative proportion of clonally rearranged to germline material observed with probes to both the immunoglobulin heavy chain and T-cell antigen receptor γ loci preclude the possibility that two distinct clones of cells are present. Identical rearrangement patterns at both blast crisis and on relapse also indicate that the same clone of cells was present on both occasions. Therefore, this strongly indicates that the HMC and the non-HMC leukemic cells in this case belong to the same clone. This finding is in contrast with the previous hypothesis that the HMC may be an intermediate step between benignity and malignancy.[15] Previous cytogenetic studies in acute lymphoblastic leukemia with HMC found that 60% had a normal karyotype and two cases showed aneuploidy invchromosomes 1 and 21.[16] Our patient had the t(9;22) translocation of chronic myelogenous leukemia, the t(8;14) chromosomal abnormality that has been identified in both T-cell and B-cell leukemias, and a t(9;15) translocation. The trisomy 8 has been identified with blast cell crises. To date, no specific chromosome abnormality has shown any relationship to HMC formation. Most cases of chronic myelogenous leukemia in lymphoblast crisis do not have HMC.

Although this case had only peripheral blood plasma for study of BaEV and SSAV, the results support previous findings of antibodies to SSAV and BaEV in bone marrow plasma of other hand mirror patients.[13] Lymphoid blast crisis in chronic myelogenous leukemia with the HMC variant requires further characterization of the cells and additional well-studied cases to determine the significance of the hand mirror cell in this disorder.

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Mixed Gonadal Dysgenesis

A Review of 15 Patients Reporting Single Cases of Malignant Intratubular Germ Cell Neoplasia of the Testis, Endometrial Adenocarcinoma, and a Complex Vascular Anomaly

Thomas M. Wallace, MD, Howard S. Levin, MD

• The clinicopathologic features of 15 patients with mixed gonadal dysgenesis are presented with special regard to cardiovascular and neoplastic disease. Seven (47%) cases, all phenotypic females, had gonadal tumors: gonadoblastoma (5), germinoma (4), malignant intratubular germ cell neoplasia (1), and a unique gonadal stromal tumor (1). Gonadoblastoma was found in 4 of 10 testes and 4 of 17 streak gonads, and associated with germinoma in 4 cases. One patient developed grade 1 endometrial adenocarcinoma after estrogen therapy. Cardiovascular diseases (ie, bicuspid aortic valve, and a unique right aortic arch with a retroesophageal arch segment, aberrant left subclavian artery, coarctation, and dissection) are documented in our series. At the time of diagnosis of mixed gonadal dysgenesis, removal of streak gonads or testes will prevent further gonadal tumor development. Cardiovascular examination may identify treatable and potentially lethal disease.

(*Arch Pathol Lab Med.* 1990;114: 679-688)

Mixed gonadal dysgenesis (MGD) is an intersex abnormality characterized by asymmetric gonadal development and was first described by Sohval in 1963.[1] In a 1973 review article, Davidoff and Federman[2] considered asymmetric gonadal development as a testis or gonadal tumor on one side with a rudimentary gonad, streak gonad, or no gonad on the other. In MGD, as a result of decreased gonadal production of müllerian inhibitory factor by the dysgenetic testis, internal müllerian structures are invariably present. The external genitalia range from normal female to normal male in appearance and often have ambiguous features. Phenotypic females often have virilization, hirsutism, and deepening of the voice at puberty. Stigmata of Turner's syndrome may be found. Most patients have a mosaic chromosome constitution that includes an XO cell line and a Y-containing cell line. Gonadal tumors arise in approximately 25% of gonadal dysgenesis patients with a Y chromosome.[3] These tumors include gonadoblastoma, germinoma, and malignant intratubular germ cell neoplasia (MIGCN).[4] Patients treated with estrogens are at risk for endometrial adenocarcinoma.[5]

Cardiovascular abnormalities in MGD have not been extensively reported. Turner's syndrome has a well-known association with aortic coarctation, bicuspid aortic valves, hypertrophic obstructive cardiomyopathy, ventricular septal defects, mitral valve prolapse, and dextrocardia.[6] In this review, we emphasize the risk of tumor development in MGD, including a unique tumor of gonadal stromal origin, and describe a unique cardiovascular anomaly characterized by a right aortic arch with a retroesophageal arch segment, an aberrant left subclavian artery, aortic coarctation, and aortic dissection.

MATERIALS AND METHODS

The files of the Medical Records Department and the Pathology Department of the Cleveland (Ohio) Clinic Foundation were searched for cases of gonadal dysgenesis from 1954 to 1989. Only gonadal dysgenesis cases with autopsy or surgical pathology material were included. The chart of each case was reviewed completely with special regard to age, clinical presentation, karyotype, cardiovascular status, neoplasms (gonadal or extragonadal), and other pertinent clinical findings. Cases of Turner's syndrome or mosaic Turner's syndrome without a Y chromosome were excluded.

The surgical pathology slides and reports of 15 cases and the autopsy slides and report of one case were reviewed. Additional sections and histochemical and/or immunohistochemical stains were ordered when appropriate. The type of each gonad (ie, streak gonad, streak ovary, or testis) was determined by

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Table 1.—Clinicopathologic Aspects of 15 Patients With Mixed Gonadal Dysgenes

Case/ Age, y/Sex†	Chief Complaint	Signs of Turner's Syndrome	External Genitalia	Müllerian Structures	Karyotype, Tissue/ Lymphocytes	Wolf Struct
1/1 y, 6 mo/M	L cryptorchidism	None	R scrotal testis, penile chordee	Rudimentary uterus, L tube	Not done	R epididy L epid L vas
2/24/M	L cryptorchidism, hypospadias	None	Hypospadias, penile chordee, R small scrotal testis	Small uterus, L tube	Not done/ 45×O,46XY	R epididy
3/6	Genital abnormality	Short stature	Clitoromegaly	L hemiuterus, vagina ends at urogenital sinus, L tube	Not done/ 46XY	R vas
14‡/F	Hirsutism‡	. . .	Male hair pattern, small right scrotal testis‡			
4/15/F	History of dysgerminoma amenorrhea	Low hair line, short fourth metacarpal	Clitoromegaly	Small uterus, bilateral tubes	46XY/46XY	None
5/15/32‡/F	Amenorrhea, vaginal bleeding‡	Short stature	Sparse pubic hair	Small uterus, bilateral tubes	Not done/ 45XO,46XYq−	L vas
6/17/F	Amenorrhea, slow growth	None	Prepubertal female	Bicornuate uterus, bilateral tubes	Not done/ 46XX,46XY	None
7/8 y, 1 mo/F	History of tumor, pain virilization	Short stature, shield chest, abnormal carrying angle	Clitoromegaly	Small uterus, bilateral tubes	45XO,46XY/ 45XO,46XY	None
8/8/F	Growth delay	Shield chest, webbed neck, short metacarpals, high palate	Prepubertal female	Small uterus, bilateral tubes	45XO,46XY/ 45XO,46XY	None
9/1 y, 1 mo/M	L cryptorchidism	None	L inguinal gonad, R scrotal testis, penile chordee	Rudimentary uterus (hernia uteri inguinale), L tube	Not done/ 45XO,46XY	R epididy L epidi
10/29/M	Retrosternal chest pain	Short stature, short metacarpals, high palate	Hypospadias, small R scrotal testis, male hair pattern	L hemiuterus, L tube	Nonviable/ 45XO,46XY 46XX	R epididy R vas
11/15/M	Ambiguous genitalia	Multiple nevi	Bifid scrotum, hypospadias, female hair pattern, no testes	Small uterus, bilateral tubes	Not done/ 45XO,46XY	R epididyr R vas
12/9 mo,	Ambiguous genitalia	None	Clitoromegaly	Small uterus, bilateral tubes	45XO,46XY/ 45XO,46XY	R epididyr
14‡/F	Abnormal sex development‡		Vaginal orifice in urogenital sinus‡			
13/18/F	Amenorrhea	None	Normal female	Normal uterus, bilateral tubes	46XY/46XY	None
14/16/F	Amenorrhea	None	Clitoromegaly	Small uterus, bilateral tubes	Not done/ 45XO,46XY	None
15/13/F	Short stature amenorrhea	Short stature	Normal female	Normal uterus, bilateral tubes	45XO,46XY/ 45XO,46XY	None

*F indicates female; M, male; R, right; L, left; DES, diethylstilbestrol; and MIGCN, malignant intratubular germ cell neoplasia.

†Sex reared as a child.

‡Second presentation.

§Identified by clinical examination only.

‖Grade 1 endometrial adenocarcinoma with focal squamous differention.

Gonads L/R	Neoplasms	Cardiovascular Renal Abnormality	Other
Inguinal streak gonad/scrotal testis[§]	None	None	Brother with cryptorchid testis
Absent/scrotal testis	None	Bicuspid aortic valve	History of schizophrenia
Abdominal streak gonad/scrotal testis	Testicular MIGCN	Anterior rotated kidney	Prematurity, retrolental fibroplasia
Abdominal testis/abdominal streak gonad	L gonadoblastoma and germinoma	None	History of allergy
Abdominal streak gonad/absent	Endometrial carcinoma[‖]	Horseshoe kidney	None
Abdominal streak gonad/abdominal streak gonad	None	None	None
Abdominal streak gonad/abdominal gonad unidentifiable	Bilateral gonadoblastoma and germinoma	None	None
Abdominal streak gonad/abdominal streak gonad	R gonadoblastoma	None	History of hypothyroidism
Inguinal streak gonad/scrotal testis[§]	None	None	None
Abdominal streak gonad/scrotal testis	None	R aortic arch, coarctation and dissection, hypertension	?DES exposure
Abdominal streak gonad/abdominal testis	None	Horseshoe kidney	None
Abdominal streak gonad/abdominal testis	None	Hypertension	Obesity
Abdominal streak gonad/abdominal streak gonad	Bilateral gonadoblastoma and germinoma	None	None
Abdominal testis/abdominal testis	L gonadoblastoma/R gonadoblastoma and germinoma	None	Cleft lip and palate
Abdominal streak gonad/abdominal streak gonad	Unclassified stromal tumor	None	None

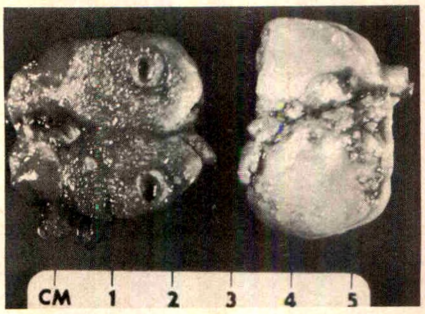

Fig 1.—Case 13. Bivalved right and left streak gonads each containing a gonadoblastoma with germinoma.

the criteria presented in the 1982 article by Robboy et al.[7]

A single case of MGD with unique cardiovascular anomalies and two cases with tumors not usually associated with MGD are presented separately.

RESULTS

Clinical Findings (Table 1)

The 15 patients ranged in age from 9 months to 29 years, with an average age of 15.8 years. Five of the patients were raised as males and 10 as females. The presentations varied and included cryptorchidism, ambiguous genitalia, amenorrhea, hirsutism, deepening voice, short stature, and/or a history of gonadal tumor. The external genitalia of the phenotypic females appeared normal in 5 cases; simple clitoromegaly was found in the other 5 cases. Either cryptorchidism (5), hypospadias (3), or both were present in the phenotypic males. Penile chordee was present in 3 cases.

Eight patients (53%) had stigmata of Turner's syndrome, which included short stature (3), shortened fourth or fifth metacarpals (3), low hair line (1), shield chest (2), short or webbed neck (3), and/or multiple cutaneous nevi (1).

Renal abnormalities documented in 3 patients included horseshoe kidney (2) and anterior rotated kidney (1).

Two patients had documented cardiovascular abnormalities: one had a bicuspid aortic valve, the other had a right aortic arch with a retroesophageal segment, an aberrant left subclavian artery, aortic coarctation, and aortic dissection. Hypertension was present in a single case.

Other miscellaneous conditions noted in the clinical history or physical exami-

Table 2.—Gonads and Associated Ductal Structures

Gonad	Total	No. % Fallopian Tube	Epididymis	Vas Deferens
Testes	10	5 (50)	6 (60)	3 (30)
Streak gonads	17	17 (100)	2 (11)	2 (12)
Aplasia	2	2 (100)	0	0
Tumor	1	1 (100)	0	0

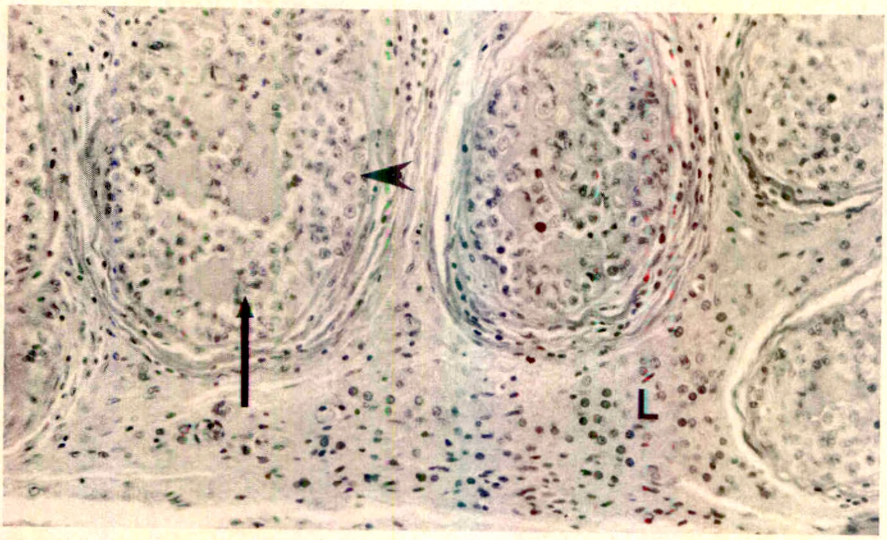

Fig 2.—Case 7. Gonadoblastoma characterized by nests of sex cord elements (arrow) and germ cells (arrowhead) with adjacent Leydig cells (L) in a streak gonad (hematoxylin-eosin, ×205).

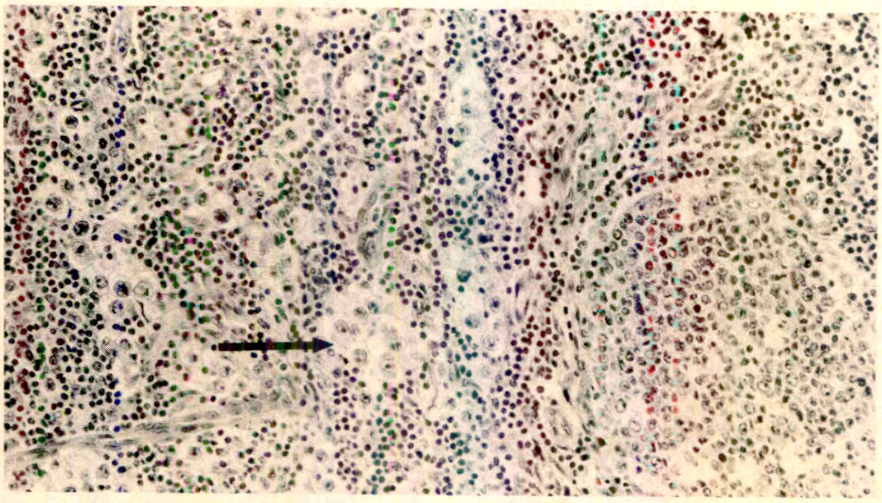

Fig 3.—Case 14. Invasive germinoma (arrow) admixed with a reactive lymphocytic infiltrate in an abdominal testis (hematoxylin-eosin, ×205).

nation were obesity (1), hypothyroidism (1), possible diethylstilbestrol exposure in utero (1), cleft lip and palate (1), schizophrenia (1), and prematurity with development of retrolental fibroplasia (1).

Pathologic Findings (Table 1)

The gonadal constitution of 7 of the patients consisted of a testis and a contralateral streak gonad. Bilateral streak gonads were found in 4 patients, while bilateral testes were present in a single case. A testis or a streak gonad with contralateral gonadal aplasia was found in 2 patients and a streak gonad with a contralateral tumor was present in a single case. In 9 patients with testes, there was a well-formed ipsilateral fallopian tube in 5 cases, and/or with a well-formed ipsilateral epididymis in 6 cases. In 2 of these 9 patients, the opposite streak gonad also was associated with an epididymis. All 17 streak gonads were associated with a fallopian tube (Table 2). Persistent müllerian structures were present in all 15 patients and ranged from a normal to an infantile to a rudimentary organ (hernia uteri inguinale, case 9). Bilateral fallopian tubes were present in 10 cases.

Gonadal tumors were found in 7 patients, all of whom were raised as females. Five patients had gonadoblastoma, of which 3 cases were bilateral (Figs 1 and 2). Invasive germinoma (Fig 3) was found associated with four gonadoblastomas. The fifth gonadoblastoma was not associated with another neoplasm. A gonadoblastoma and/or germinoma was present in 3 of 10 testes, 4 of 17 streak gonads, and in one case without an identifiable gonad. Single cases of MIGCN in a scrotal testis, unclassified stromal tumor in a streak gonad, and endometrial adenocarcinoma with squamous differentiation were identified.

The gonadal stromal tumor presented in a 13-year-old girl with feminizing characteristics such as breast buds, and axillary and pubic hair. Cut surface revealed a bright yellow and homogeneous nodule. On hematoxylin-eosin section, the tumor was composed of plump spindled cells arranged in sheets and occasional nests without tubule formation. The nuclei showed slight atypia and contained small nucleoli. Mitoses were prominent (average 11 mitoses per 10 high-power fields) (Fig 4). The atypia and mitoses were worrisome features in regard to potential malignancy. She was alive and well 10 months after surgery. This case will be described in greater detail after a suitable follow-up period to better ascertain the behavior of this tumor (T.M.W., Biscotti C.V., MD, and Hart W.R., MD, unpublished data, March 1990).

Chromosomal analysis of blood lymphocytes, gonadal tissue, and/or skin fibroblasts was attempted in 14 cases. Eleven patients had mosaic karyotypes, which included 45,XO/46,XY (9), 46,XX/46,XY (1), and 45,XO/46,XX/46,XY (1). Three patients had a nonmosaic 46, XY karyotype. Y chromosomes were present in all 14 patients who underwent karyotyping. Six patients had karyotypes of both tissue and blood lymphocytes. In each case, the paired karyotypes were identical.

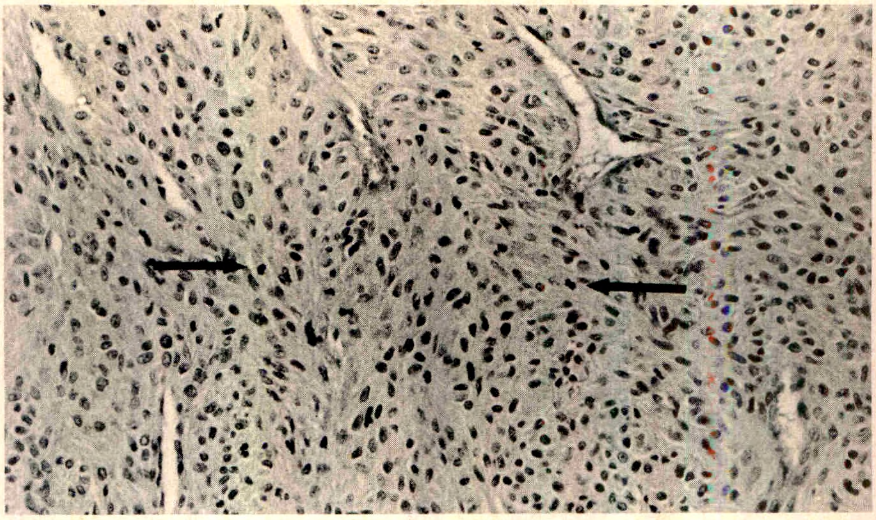

Fig 4.—Case 15. Stromal tumor in a streak gonad. Sheets of spindled to polygonal cells containing lipid and having numerous mitoses (arrows) characterize the tumor (hematoxylin-eosin, × 205).

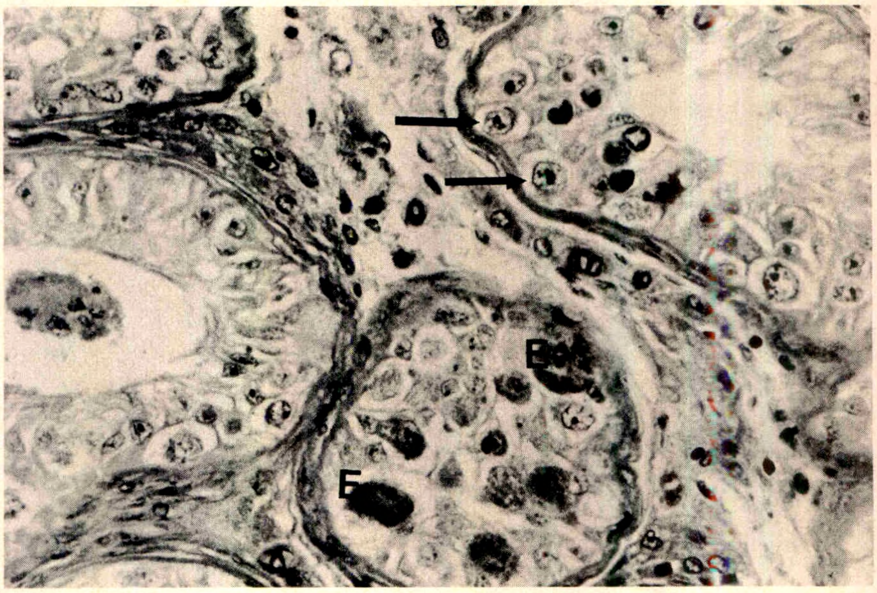

Fig 5.—Case 3. Testicular malignant intratubular germ cell neoplasia characterized by enlarged hyperchromatic germ cells with large nucleoli (arrows) confined to the seminiferous tubule. Several Sertoli cells with eosinophilic granular change (E) are identified (hematoxylin-eosin, × 333).

Case 3.—A 36-year-old woman first presented to the Cleveland Clinic Foundation at the age of 6 years for genital abnormalities. Her medical history was significant for prematurity (1.6 kg), retrolental fibroplasia, and clitoromegaly. Physical examination revealed a short phenotypic female with clitoromegaly and underdeveloped labia majora. The labia minora were absent. Subsequently, a clitorectomy was performed. At the age of 14 years, she complained of facial hair, a deepening voice, and masculine physique. Pelvic examination at this time was remarkable for a palpable right scrotal testis, small uterus, and adnexal structures. Cystoscopic examination revealed a small urogenital sinus into which the urethra and vagina opened. The peripheral blood lymphocyte karyotype was 46,XY. Subsequently, a total abdominal hysterectomy, left salpingogonadectomy, and right orchiectomy were performed. A small hemiuterus, fallopian tube, and left streak gonad were identified. The majority of the testicular parenchyma consisted of closely apposed seminiferous tubules, with intertubular diffuse Leydig cell hyperplasia. The majority of tubules contained only mature Sertoli cells; occasional Sertoli cells had oxyphilic granular change. Other tubules near the tunica albuginea contained many large hyperchromatic germ cells with large nucleoli. Periodic acid–Schiff-positive diastase-sensitive material was present within the cytoplasm of the germ cells. Immunohistochemical staining for placental alkaline phosphatase could not be performed because the tissue had been fixed in Zenker's solution. These germ cells were confined to the tubules, did not invade the interstitium, and were typical of MIGCN (Fig 5). The patient was alive and well without evidence of malignancy 22 years after surgery.

Case 5.—A 36-year-old woman presented at the age of 15 years for amenorrhea. Her history was remarkable for a horseshoe kidney. Physical examination revealed a short phenotypic female with sparse pubic hair and underdeveloped breasts. Karyotypic analysis of peripheral blood lymphocytes demonstrated a 45,XO/46,XYq$^-$ pattern.

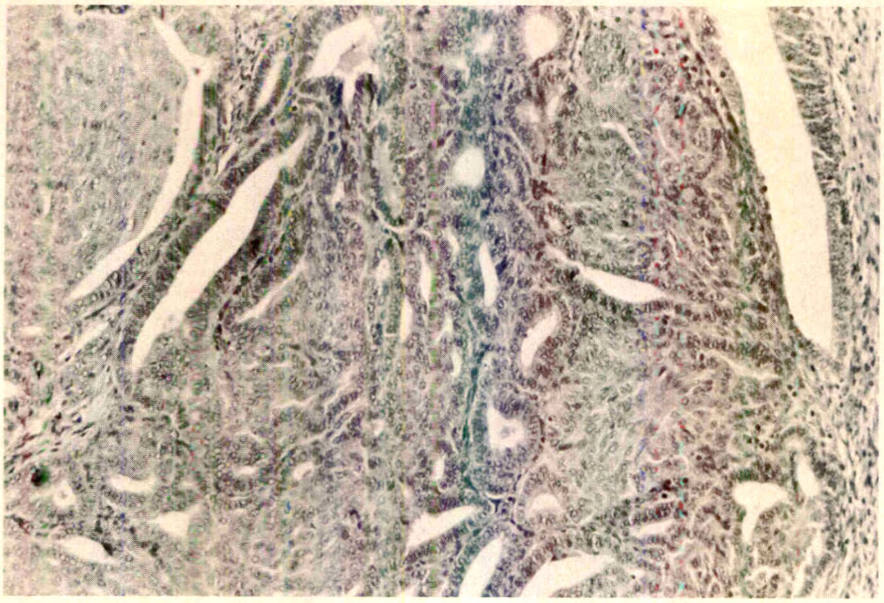

Fig 6.—Case 5. Grade 1 endometrial adenocarcinoma characterized by back-to-back glands without intervening stroma and with focal squamous differentiation (hematoxylin-eosin, ×131).

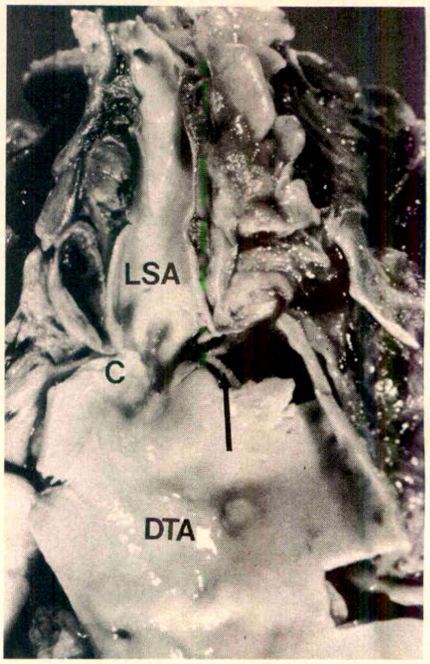

Fig 7.—Case 10. Autopsy specimen of aortic arch viewed from the left lateral position. The left subclavian artery (LSA), descending thoracic aorta (DTA), coarctation (C), and prosthetic graft (arrow) are identified.

Conjugated estrogens (1.25 mg) were taken in a cyclic fashion for 6 years. Increased breast size and regular menses were noted. At the end of 6 years, she noticed heavier menses and irregular spotting. Medroxyprogesterone acetate (5 mg) was added at the end of the cyclic estrogen regimen for the next 18 months but spotting continued. An endometrial biopsy revealed a grade 1 adenocarcinoma with squamous differentiation; a total abdominal hysterectomy, bilateral salpingectomy, and left gonadectomy were subsequently performed. No right gonad was identified.

The uterus measured 7.5×5.0×3.0 cm and was pear shaped. The cervical os was unremarkable. The endometrium appeared shaggy and thickened, measuring 0.9 cm. Grossly, no myometrial invasion was found. Microscopically, a grade 1 adenocarcinoma with squamous differentiation was identified (Fig 6) in the endometrium with no invasion of the myometrium. Cystic, adenomatous, and atypical hyperplasia were present in different sections adjacent to the adenocarcinoma. Postoperatively, she continued to take conjugated estrogens and medroxyprogesterone acetate. She was alive and well with no evidence of malignancy 15 years after surgery.

Case 10.—A 29-year-old phenotypic man had a 3-month history of hypertension. As a child, he underwent many penile surgeries for correction of hypospadias. While driving, he experienced severe chest pain and dyspnea. On arrival at an emergency department, his blood pressure was 150/100 mm Hg. A chest roentgenogram showed a widened mediastinum and a subsequent aortogram revealed a dissection of a right aortic arch. The dissection originated at the left subclavian artery and extended anterograde to the L2 vertebral level, typical of type 3 aortic dissection. The great vessels originated from the arch as follows (proximal to distal): left common carotid, right common carotid, right subclavian artery, and last the left subclavian artery. The aortic arch coursed behind the esophagus to a left-descending thoracic aorta. He was transferred to the Cleveland Clinic Foundation. A continuous nipride drip controlled his hypertension but several days later he experienced mental status changes. A computed tomographic scan of the head was unremarkable. An echocardiogram showed only left ventricular hypertrophy and normal valves. Subsequently, he suffered several apneic episodes. Intubation at this time was difficult due to subglottic tracheal stenosis. A retrograde dissection of the aortic arch was postulated as the cause of the external tracheal compression. He underwent median sternotomy and the retrograde dissection was confirmed. A graft was placed from the uninvolved ascending aorta to the proximal descending thoracic aorta. The great vessels of the neck were individually anastomosed to the graft. Uncontrolled hemorrhage from the distal anastomosis led to cardiovascular collapse and exsanguination.

The postmortem external examination revealed a young man measuring 160.0 cm in height with full beard and mustache. The neck was short and thick but not webbed. The median sternotomy was identified in an otherwise normal-appearing chest. The fourth and fifth metacarpals of both hands and the left fourth metatarsal bones were shortened. He had a male genital hair pattern. The penis measured 5.0 cm in length with evidence of remote hypospadias repair. A small soft testis was palpated in the right hemiscrotum. No vaginal opening was identified.

The aortic arch graft originated at the uninvolved ascending aorta, coursed behind the esophagus, and was sewn to the descending left thoracic aorta. The distal anastomosis was partially sewn to

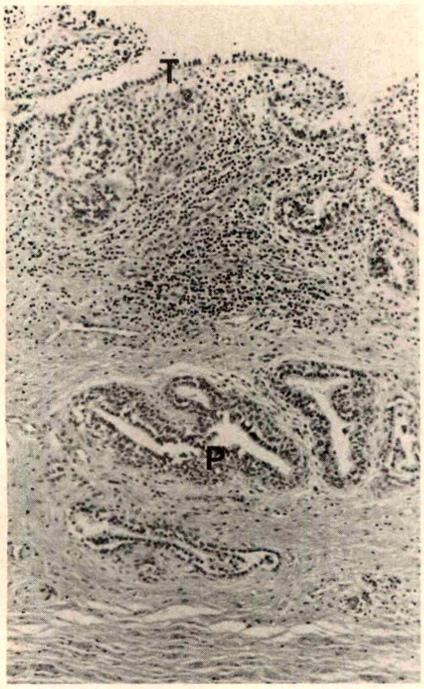

Fig 8.—Case 10. Urinary bladder neck demonstrating rudimentary prostatic glands (P) characterized by double-layered cuboidal to columnar epithelium. Immunohistochemical stains for prostate specific antigen and prostatic acid phosphatase revealed staining of the glands but not of the overlying transitional epithelium (T)(hematoxylin-eosin, × 103).

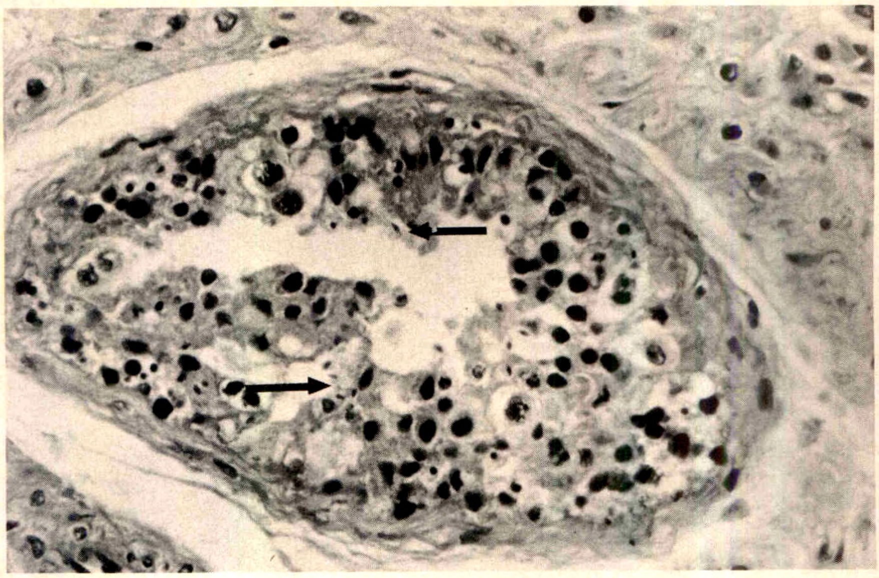

Fig 9.—Case 10. Lancet-shaped late spermatids (arrows) in selected testicular tubules (hematoxylin-eosin, × 333).

the false wall of the dissection. The great vessels of the neck were individually sewn to the graft in the order shown by the arteriogram. The anastomoses were intact. The dissection extended anterograde to the L2 vertebral level and involved the left subclavian and superior mesenteric arteries. A small coarctation was found at the level of the nonpatent ductus arteriosus, which connected the left pulmonary artery and the aorta (Fig 7). The remainder of the aorta was mildly atherosclerotic. No congenital cardiac abnormalities were noted. A recent and remote left ventricular myocardial infarct was present. The coronary arteries were mildly atherosclerotic.

A small left-sided unicornuate uterus lay between the urinary bladder and the rectum. A well-developed fallopian tube was present on the left. At the distal end of the tube, a small streak gonad was present. The uterine cervix projected into a small vagina which ended blindly at the perineal skin. The right scrotal testis was small, soft, and tan-brown, and associated with a well-formed epididymis and vas deferens. No testis or wolffian duct remnants were noted on the left. No grossly identifiable prostate or seminal vesicles were found. The kidneys and collecting systems were unremarkable.

Histologic examination of the bladder neck was significant for focal small glands in the lamina propria beneath the transitional epithelium (Fig 8). The glands consisted of doubled-layered cuboidal to columnar cells with basilar bland nuclei. Immunohistochemical stains were positive for prostate specific antigen and prostatic acid phosphatase, indicating rudimentary prostate tissue. The small uterus had an organized smooth-muscle wall lined by atrophic endometrium with extensive squamous metaplasia. The cervix and vagina were lined by chronically inflamed squamous mucosa. The left streak gonad consisted solely of ovarian-type stroma with occasional primitive sex cords. Rete ovarii were present in the hilus. Hilus cells were prominent surrounding small nerves. No ova were present. The right testis displayed prominent aggregates of Leydig cells in the interstitium and marked tubular sclerosis throughout the testis. Several tubules exhibited complete spermatogenesis with identifiable mature spermatids (Fig 9). Focal areas of ovarian-type stroma were seen in the tunica albuginea. Neoplastic cells were not seen in either gonad. The right epididymis and right vas deferens were remarkable for identifiable spermatozoa in their lumina.

Blood lymphocytes and skin fibroblasts were cultured for chromosome analysis. The fibroblasts were nonviable but the lymphocyte culture revealed a mosaic 45,XO/46,XY/46,XX karyotype.

COMMENT

In 1963, Sohval[1] coined the term *mixed gonadal dysgenesis* for a group of intersex patients with asymmetric gonadal development. The gonadal complement included the following: (1) a testis on one side and a contralateral streak gonad; (2) a testis and contralateral gonadal agenesis; (3) hypoplastic gonads with rudimentary tubules in one; or (4) a streak gonad with a contralateral tumor. Davidoff and Federman[2] considered a testis or gonadal tumor on one side and a rudimentary gonad, streak gonad, or no gonad on the other side as MGD. Because of insufficient or delayed production of müllerian inhibitory factor by the dysgenetic testis, müllerian structures such as a uterus or fallopian tube are invariably present. Some patients may present with hernia uteri inguinale (müllerian structures such as a uterus or fallopian tube in an inguinal hernia). The majority of cases have bilateral fallopian tubes despite the presence of a testis. Inadequate or delayed production of testosterone by the dysgenetic testis causes incomplete masculinization of the external genitalia and poor development of the ipsilateral wolffian duct structures. The external genitalia range from normal female to normal male in appearance. Most patients present at birth with ambiguous genitalia and two of three are raised as females. At puberty, the patients often develop hirsutism, deepening of the voice, and other signs of virilization. Stigmata of Turner's syndrome may be present in one of three patients and include low hair line, shield chest, multiple pigmented nevi, webbed neck, and cubitus valgus. Half of MGD patients are taller than 147 cm and usually do not display signs of Turner's syndrome. The 45,XO/46,XY mosaic karyotype is the most common, followed by 46,XY and less frequently other mosaic karyotypes with a Y-chromosome-containing cell line. In patient 10, the 45,XO/46,XX/46,XY peripheral lymphocyte karyotype may not have been accurate due to the blood transfusions he received during surgery. All six units were donated by women. Prince and Ahrens[8] have demonstrated that lymphocytes remain viable and stable for at least 14 days in stored blood. Thus, the 46,XX cell line may not represent a cell line in our patient and the true karyotype may be 45,XO/46,XY. The possibility of a female donor being a patient with Turner's syndrome or having a 46,XY cell line is remote.

The testis of adult patients with MGD usually shows no spermatids or spermatozoa and is associated with infertility in almost all patients. Rajfer and Walsh[9] stated that the postpubertal testis lacks advanced stages of spermatogenesis, has immature Leydig cells, and commonly has hyalinization and thickening of the tubular basement membrane with interstitial fibrosis. In our postpubertal patients, the seminiferous tubules ranged from totally sclerotic to tubules with only mild hypospermatogenesis. Both patients 2 and 10 revealed mature spermatids within the seminiferous tubules of descended testes. Patients 3 and 11 demonstrated tubules with only mild tunica proprial thickening and germ-cell aplasia. Several oxyphilic granular Sertoli cells were present in these 2 patients, 1 of whom was cryptorchid. Wong et al[10] showed that these granules are secondary lysosomes containing membrane fragments, phospholipid, and amorphous material. All of the postpubertal testes had prominent Leydig cells. On the other hand, the abdominal testis of the prepubertal phenotypic female (patient 12) revealed closely apposed immature tubules with scattered germ cells. Sclerotic tubules or interstitial fibrosis were not seen. Leydig cells were not present. The tunica albuginea may contain ovarian-type stroma similar to that in a streak gonad. A streak gonad consists of ovarian-type stroma but has no primordial ovarian follicles. Streak ovaries, streak gonads with primordial follicles, or germ cells, were not found among our patients. Poorly differentiated sex cords may be found in the stroma with hilus cells adjacent to nerves in the hilus of the streak gonad.

Dysgenetic male pseudohermaphroditism (DMPH) is a disorder characterized by bilateral dysgenetic testes, persistent müllerian structures, cryptorchidism, and inadequate virilization. XO/XY mosaicism may be found. In adulthood, spermatogenesis and fertility are invariably absent. Rajfer et al[11] consider DMPH to be closely related to MGD. Pure gonadal dysgenesis (PGD), on the other hand, is characterized by bilateral streak gonads, internal müllerian structures, and female phenotype with no signs of Turner's syndrome.[12] Both Robboy et al[7] and Rajfer and Walsh[9] consider PGD and DMPH to be within the spectrum of MGD. Each syndrome has similar features: ambiguous genitalia, persistent müllerian duct structures, occasional wolffian duct derivatives, karyotypes having a Y chromosome, and the potential for gonadal neoplastic transformation. We agree that MGD includes a spectrum of patients with bilateral testes, a testis and a streak gonad, bilateral streak gonads, or gonadal tumors.

In our series, 67% of patients were reared as females. Three of the phenotypic males had hypospadias and all presented with at least unilateral cryptorchidism. Slightly more than half of the patients displayed stigmata of Turner's syndrome, which included short stature, shortened fourth and fifth metacarpals, low hair line, shield chest, short or webbed neck, and multiple cutaneous nevi. All had an identifiable uterus and 66% also had bilateral fallopian tubes even if a testis was present. Most karyotypes were mosaics (78%), the most frequent being 45,XO/46,XY (9). The nonmosaic karyotypes were 46,XY. These characteristics of our patient group are typical of other published series or case reports of MGD.[2,7,13-16]

Cardiovascular abnormalities in MGD have not been extensively reported in the literature. Turner's syndrome, on the other hand, has a well-known association with cardiovascular anomalies, including aortic coarctation, congenital bicuspid aortic valve, hypertrophic obstructive cardiomyopathy, ventricular septal defect, mitral valve prolapse, and dextrocardia. Of Turner's syndrome patients with a 45,XO karyotype, 30% to 50% exhibit some of these abnormalities; 70% are coarctation. Fewer anomalies are seen in Turner's syndrome mosaicism (XO/XX).[6] In reviewing the published series of MGD that included comments on cardiovascular diseases, aortic coarctation,[16,17] congenital heart disease,[13] and hypertension[13] have been noted. In our series, hypertension, bicuspid aortic valve, and the right aortic arch with a retroesophageal segment, an aberrant left subclavian artery, aortic coarctation, and dissection were found. Right aortic arches were classified by Shuford et al[18] as being associated with (1) an aberrant left subclavian artery, (2) mirror image branching, or (3) isolation of the left subclavian artery. The most common type is the right aortic arch with an aberrant left subclavian artery, present in 0.05% of the population.[19] Knight and Edwards[20] subdivided cases of right aortic arch according to the presence or absence of a retroesophageal arch segment. The mirror image and the aberrant left subclavian type without a retroesophageal segment are often found with congenital heart diseases such as tetralogy of Fallot, deficiency of the ventricular septum, and tricuspid atresia. As in our case, the right aortic arch with an aberrant left subclavian artery and a retroesophageal segment is rarely found with congenital heart disease. The combination of a coarctation and right aortic arch is rare and only a few cases have been reported,[21-23] one being in a patient with Turner's syndrome.[24] Dissection of a right aortic arch is also rare, but a few case reports exist.[25-27] More often, dissection of a left aortic arch is seen in patients with Turner's syndrome.[28-32] We found no other cases of a right aortic arch with an aberrant left subclavian artery, coarctation, and dissection, as shown in our patient 10. Complete cardiovascular examination of patients with gonadal dysgenesis may reveal treatable and potentially lethal disease.

The frequency of gonadal tumor development has been stressed in the literature. Gonadal tumors develop in approximately "30% of patients with 46,XY PGD, from 9% to 25% of those with MGD and in up to 30% of those with DMPH."[33] Manuel et al[34] calculated the expectancy of development of a gonadal tumor in PGD by age 10 years to be 2%, by age 20 years to be 16%, and by the age of 30 years to be 27.5%. Ten percent of MGD patients may also develop gonadal tumors by age 20 years and 19% by age 30 years. The most common tumor in MGD is the gonadoblastoma, which consists of nests of germ cells similar to a seminoma or dysgerminoma in relationship to cells with small oval nuclei and inconspicuous cytoplasm resembling immature Sertoli or granulosa cells (sex cord derivatives). Elements indistinguishable from Leydig cells are present in approximately two of three cases. Calcification and hyalinization occur often. Gonadoblastoma is bilateral in one of three cases and half are overgrown by an invasive germinoma. Patients with gonadoblastoma and metastatic germinoma have an overall favorable prognosis after surgery and radiation. Other associated malignant germ-cell elements such as teratoma, embryonal carcinoma, yolk sac tumor, or choriocarcinoma may be present. With the exception of those with teratoma, most patients with gonadoblastoma and malignant germ-cell tumors have developed metastases and died shortly thereafter.[35,36] Adawole et al[37] have reported a patient with gonadoblastoma, dysgerminoma, and metastatic choriocarcinoma who was treated with chemotherapy and had no evidence of disease at the time of publication. Approximately four of five gonadoblastomas arise in phenotypic females; 20% of tumors arise in a streak gonad, 20% in a testis, and in the rest of the cases, the gonad cannot be identified.[33,38] The second most common tumor in MGD is the pure germinoma. These have probably overgrown a gonadoblastoma or testicular MIGCN. Testicular MIGCN has been described in patients with 45,XO/46,XY mosaicism.[4] Juvenile granulosa cell tumor has been reported in a dysmorphic infant,[39] and in gonadal dysgenesis patients with a Y chromosome.[40-42] Wilms' tumor has been associated with MGD, PGD, DMPH,[43,44] and the Drash syndrome,[45] and a single case of Wilms' tumor was associated with gonadal dysgenesis, Fanconi's anemia, medulloblastoma, and horseshoe kidney.[46] Rajfer[43] believes that a common defect in the urogenital ridge, the embryonic precursor of the kidney, and the gonads, may be the cause of the association of gonadal dysgenesis and renal tumors. Single-case reports of MGD patients with a peritoneal mesothelioma[47] and "serous cystadenocarcinoma" of an unidentified gonad[48] are present in the literature. Adenocarcinoma of the endometrium has been described in patients with Turner's syndrome treated with estrogens.[5,49,50] Two thirds of the reported cases had squamous differentiation, as in our patient 5.[49-51] Krishnamurthy et al[50] recommend close follow-up of all patients with Turner's syndrome treated with estrogens and progesterone because apparently "progestins do not always exert a protective effect against estrogen-stimulated carcinogenesis." An estrogen-associated endometrial osteogenic sarcoma has been identified in a 28-year-old woman with Turner's syndrome after 16 years of estrogen-progesterone therapy.[52] Single cases of MGD patients with endometrial adenocarcinoma were included in two series.[5,7] Thus, close follow-up of patients treated with estrogen, with or without progesterone, is recommended.

In our series, 47% of patients developed gonadal tumors, which is slightly higher than other series. All tumors arose in phenotypic females and 60% of the gonadoblastomas were bilateral. Germinomas were found in 75% of the gonadoblastomas. The gonadal stromal tumor is unique and will be reported separately after a suitable follow-up period. Khoo and Buntine[53] have described a "malignant lipid-type sex cord stromal tumor" in a streak gonad of a mosaic 46,XX/47,XXX female. Lyons et al[54] described a "hilus cell tumor" in a streak gonad of a virilized patient with Turner's syndrome. In addition to the gonadoblastoma and germinoma, we reported single cases of testicular MIGCN and an endometrial adenocarcinoma occurring after long-term estrogen/progesterone therapy. Our series highlights the malignant potential of the dysgenetic gonad (usually germ cell and rarely sex cord-stromal tumors) in MGD and emphasizes the risk of endometrial adenocarcinoma following exogenous estrogen therapy.

Gonadal extirpation at the time of diagnosis is recommended in patients with MGD, since malignant gonadal tumors can develop in childhood. Siebenmann[55] and Aarskog[56] have stated that a scrotal testis in a child with MGD or DMPH may be left in place, since malignant tumors have not occurred in a scrotal testis. However, the testicular MIGCN in our series was found in the scrotal testis of a 14-year-old. Müller et al[4] recommended testicular biopsy of all XO/XY gonadal dysgenesis cases with a male phenotype as soon as the syndrome is diagnosed. Then, if MIGCN is found, a gonadectomy is warranted.

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Morphometric Analysis of Germinal Centers in Nonthymomatous Patients With Myasthenia Gravis

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• A morphometric analysis of germinal centers (GCs) in the thymus of nonthymomatous patients with myasthenia gravis was performed in 26 patients, including 14 women and 12 men. The GCs were evaluated for number, diameter, perimeter, and cross-sectional area using a computerized interactive morphometric analysis. The GCs ranged from 2 to 19 per case, with an average diameter of 0.02 to 0.43 mm, a perimeter of 0.38 to 1.35 mm, and a cross-sectional area of 0.01 to 0.14 mm^2. In thymic sections with fewer than five GCs, the GCs had a mean area of 0.05 mm^2, a perimeter of 0.84 mm, and a diameter of 0.24 mm. In thymic sections containing five or more GCs, the GCs had a mean area of 0.04 mm^2, a perimeter of 0.62 mm, and a diameter of 0.19 mm. The clinical improvement in these patients inversely correlated with the above parameters, with improvement being observed in patients whose GCs had a mean area of 0.02 mm^2, a perimeter of 0.58 mm, and a diameter of 0.17 mm. No correlation was observed between the number of GCs and clinical improvement. The results of our study appear to indicate that computerized interactive morphometric analysis may represent a potentially useful method for the evaluation of the morphometric features of thymic GCs and their correlation with clinical outcome in patients with myasthenia gravis.

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Myasthenia gravis is a neuromuscular disorder manifested by weakness and fatigability of voluntary muscles. Although the thymus gland has long been suspected to play a role in the development of this disorder,[1-3] its relationship with myasthenia gravis is still not well understood. Favorable results following thymectomy, however, have provided evidence in favor of such an association.[4-7]

The morphological hallmark of the thymus in nonthymomatous patients with myasthenia gravis is the presence of germinal centers (GCs). This feature has been used as a parameter to predict the clinical outcome in these patients. However, conflicting results have been obtained by conventional morphometric studies, with some studies claiming a better clinical outcome in patients with numerous GCs,[8-9] while others have demonstrated the contrary.[10-11]

We evaluated the use of computerized interactive morphometric analysis of GCs in the thymuses of patients with nonthymomatous myasthenia gravis. The advantages of this methodology over other conventional morphometric analyses is discussed.

PATIENTS AND METHODS

Twenty-six patients with myasthenia gravis who were surgically treated at The Mt Sinai Hospital, New York, NY, during the years 1975 through 1976 were studied. The clinical features of these patients are shown in Table 1. A clinical follow-up of at least 8 years was available for all the patients in this study. All the patients were treated with anticholinesterases and thymectomy. The anticholinesterase treatment in 24 patients varied from 1 to 9 years. Two patients (Nos. 16 and 22) received treatment for more than 10 years. Only one patient (No. 16) received combination treatment with anticholinesterases and steroids. All of the patients were preoperatively classified according to the Osserman[12] classification. Clinical response to thymectomy was assessed by physical examination and electromyographic studies in all of the patients. Reclassification in all patients was performed according to the same method used preoperatively. Eleven patients showed clinical improvement at follow-up. Of these, 8 were found to be in remission and 3 were found to have improved from generalized mild disease (IIA) to mild ocular symptoms or relatively free of symptoms. Fifteen patients showed no signs of improvement as judged by the results of electromyographic studies and physical examination.

All available hematoxylin-eosin–stained sections (ranging from one to eight per case) were studied, and the GCs were traced, producing a quantitative analysis of the number, perimeter, diameter, and cross-sectional area, using a computerized interactive morphometric analysis. This technique utilizes a 33-cm (13-in) monitor overlaid with a high-resolution touch-sensitive panel; video images generated by a high-resolution black-and-white video camera mounted on top of a microscope (Leitz Orthoplan) are directly traced on the touch panel.[13] The method is very efficient, allowing a quick tracing of GCs with computer storage, computation, and generation of analytic data.[14] We emphasize that this approach is interactive (ie, the structure recognition and the tracing are done by the investigator rather than the machine) to obviate the uncertainties caused by the lack of specificity of gray levels and instrumental structure recognition and boundary tracing that depend on it. With the interactive approach, the level of effort required is minimal.

We utilized all thymic sections available. The number of sections for each case varied

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Reprints not available.

Table 1.—Clinical Features in 26 Patients With Nonthymomatous Myasthenia Gravis*

Patient No./ Sex/Age at T, y	Classification at Onset†	Treatment	Follow-up†
1/F/47	IIA	AC+T	IIA
2/F/18	IIA	AC+T	IIA
3/F/35	IIA	AC+T	IIA
4/F/39	IIA	AC+T	IIA
5/M/34	IIB	AC+T	IIB
6/F/21	IIA	AC+T	IIA
7/F/30	IIA	AC+T	IIA
8/F/20	IIA	AC+T	IIA
9/M/58	IA	AC+T	IA
10/M/17	IIA	AC+T	IIA
11/F/31	IIA	AC+T	IIA
12/M/33	I	AC+T	IIA
13/M/12	I	AC+T	IIA
14/M/48	I	AC+T	IIA
15/F/22	IIA	AC+T	IIA
16/M/45	IIA	AC+S+T	I
17/F/24	IIA	AC+T	Rem
18/F/28	IIA	AC+T	Rem
19/M/34	IIA	AC+T	Rem
20/M/30	IIA	AC+T	Rem
21/M/54	IIA	AC+T	Rem
22/F/47	IIA	AC+T	I
23/F/37	IIA	AC+T	Rem
24/F/26	IIA	AC+T	Rem
25/M/73	IIA	AC+T	I
26/M/27	IIA	AC+T	Rem

*T indicates thymectomy; AC, anticholinesterases; S, steroids; and Rem, remission.

†Classification according to Osserman.[12]

Table 2.—Morphometric Features in 26 Patients With Nonthymomatous Myasthenia Gravis

Patient No.	No. of Germinal Centers	Area, mm²	Perimeter, mm	Diameter, mm
1	7	0.07	0.09	0.31
2	3	0.06	0.87	0.28
3	2	0.07	0.96	0.31
4	2	0.08	1.04	0.33
5	11	0.05	0.75	0.24
6	4	0.03	0.60	0.19
7	2	0.04	0.79	0.25
8	5	0.06	1.18	0.38
9	5	0.04	0.70	0.22
10	3	0.14	1.35	0.43
11	5	0.07	0.52	0.02
12	10	0.01	0.42	0.13
13	9	0.04	0.78	0.25
14	2	0.03	0.63	0.20
15	3	0.07	0.95	0.30
16	3	0.04	0.72	0.23
17	3	0.03	1.11	0.18
18	4	0.04	0.74	0.24
19	5	0.02	0.49	0.16
20	4	0.02	0.70	0.22
21	2	0.01	0.38	0.12
22	8	0.01	0.39	0.12
23	19	0.03	0.93	0.03
24	9	0.03	0.71	0.22
25	5	0.01	0.41	0.13
26	6	0.01	0.42	0.13

from one to eight. On each section, every single identifiable GC was traced. The computer was programmed to extract from each tracing the following data: cross-sectional area (in square millimeters), perimeter (boundary line between GC and dense lymphocytic tissue, in millimeters), and largest diameter of the above (in millimeters). This work does not address the question of the dimensions of the GCs or the three-dimensional space, which did not appear necessary for the purposes of this work, since the service pathologist makes the diagnosis by study of flat sections. The question of interobserver and intraobserver variations with our particular system has been previously addressed.[15] No issue of random sampling arose, since we measured all the GCs found in all the sections studied.

The clinical improvement of these patients was correlated with the results of the morphometric analysis using a general linear model procedure.

RESULTS

The results of the morphometric analysis on GCs are shown in Table 2. The total number of GCs found in each case ranged from 2 to 19 (median, 5). The diameter of the GCs ranged from 0.02 to 0.43 mm, with a perimeter of 0.38 to 1.35 mm and a cross-sectional area of 0.01 to 0.14 mm². In thymic sections containing fewer than five GCs, the GCs had a mean cross-sectional area of 0.05 mm², a perimeter of 0.84 mm, and a maximum diameter of 0.24 mm. In thymic sections with five or more GCs, the GCs had a mean cross-sectional area of 0.04 mm², a perimeter of 0.62 mm, and a diameter of 0.19 mm.

The results of the analysis of variance using a general linear model procedure showed a statistical significance of .01 when the clinical improvement of the patients was correlated with the cross-sectional area of the GCs. A significance of .05 was observed between the perimeter and the diameter of the GCs and the clinical improvement of the patients. No correlation was obtained between the number of GCs and the clinical improvement in these patients.

COMMENT

Since the first study of GCs in thymuses of patients with myasthenia gravis,[16] there has been an increasing interest in correlating the presence or absence of GCs as a predictor of clinical outcome for these patients. The results of these studies have been, for the most part, inconclusive. Castleman and Norris,[17] in their study in 1949, found no correlation between the changes in the thymus and the response to thymectomy. In 1968 Mackay et al[18] found that young female patients with numerous GCs responded better to thymectomy. However, in 1971 Alpert et al[19] noted that patients with few or no GCs also showed a good response.

Studies using semiquantitative analyses have been performed in the past, such as the one by Alpert et al.[19] Their study, however, also included cases of thymomas in patients with myasthenia gravis. Reinglass and Brickel[20] performed a semiquantitative study that included mostly young female patients. This study failed to find any correlation between the results of thymectomy and the morphological appearance of the thymus. The study performed by Mackay et al[18] lacked a longer follow-up period and, contrary to our study, was not limited to nonthymomatous patients with myasthenia gravis. Vetters and Simpson,[21] using histologic quantitation methods, made a comparison of the histologic characteristics of the thymus with the response of thymectomy in patients with myasthenia gravis. Their study found a tendency for patients with unreactive thymuses to do better after thymectomy.

In the present study, we have attempted to evaluate the use of a computerized morphometric analysis in the study of the dimensions of GCs in the thymus of patients with nonthymomatous myasthenia gravis. The results of our study indicate that there is little correlation between the number of GCs and their cross-sectional area, perimeter, and diameter. In thymic sections containing fewer than five GCs, the GCs had a mean cross-sectional area of $0.05\ mm^2$, a perimeter of 0.84 mm, and a diameter of 0.24 mm, whereas in those with five or more GCs, the GCs had a mean cross-sectional area of $0.04\ mm^2$, a perimeter of 0.62 mm, and a diameter of 0.19 mm. The overall cross-sectional area of reactive thymic tissue in patients with myasthenia gravis may thus be greater than is apparent even though there are fewer GCs. This finding may have functional implications and may perhaps explain some of the conflicting results previously reported in the literature concerning the correlation of the number of GCs to the clinical response in patients with myasthenia gravis. The same parameters in our study, when correlated with the clinical evolution of our patients, showed that in those patients with clinical improvement the overall dimensions of the GCs showed a mean cross-sectional area of $0.02\ mm^2$, a perimeter of 0.58 mm, and a diameter of 0.17 mm, whereas in those who did not show signs of improvement the GCs had a mean cross-sectional area of $0.06\ mm^2$, a perimeter of 0.82 mm, and a diameter of 0.25 mm. An analysis of variance using a general linear model procedure demonstrated a significance of .01 between the surface area of the GCs and the clinical improvement obtained after an 8-year follow-up period, and a significance of .05 between the perimeter and the diameter of the GCs and the clinical improvement of these patients. The number of GCs in our cases did not bear any significant correlation with clinical outcome.

The discrepancy observed between the number of GCs and the overall dimensions of these structures in this series suggests that determination of the number of GCs alone may not be a reliable prognostic parameter for the evaluation of patients with myasthenia gravis. The inclusion of additional variables such as the cross-sectional area, the perimeter, and the diameter of GCs may provide additional valuable information that could be of significance in the evaluation of these patients. Moreover, morphometric analysis of GCs by computerized image represents a more objective tool and one that is amenable to statistical analysis, which will serve to provide more specific, quantitative data with which to evaluate the morphological features of the thymus in these patients.

Although the number of patients in this study is too small to draw any definitive conclusions, the preliminary results presented in this study would seem to indicate that computerized interactive morphometric analysis of GCs may constitute a valuable technique for studying the role of the morphological features of thymus in patients with myasthenia gravis. Additional prospective studies using this method in a larger number of patients would be of interest to determine whether a significant clinical correlation exists between dimensions of GCs and prognosis in this condition.

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Granular Cell Tumor

Immunohistochemical Analysis of 21 Benign Tumors and One Malignant Tumor

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• We examined the immunohistochemical profile of 21 granular cell tumors (GCTs) and a single clinically malignant GCT using a panel of commercially available antibodies. All cases showed diffuse cytoplasmic and nuclear staining for S100 protein. Fourteen cases stained for myelin basic protein, Leu-7, or both. Immunostains for neurofilament protein and glial fibrillary acidic protein were negative in all cases. Stains for cathepsin B and α_1-antichymotrypsin were positive in 21 and 15 cases, respectively. Cathepsin-B reactivity may reflect autodigestion of myelin, while the presence of α_1-antichymotrypsin is less specific and may be related to cellular production of this product or to nonspecific uptake of α_1-antichymotrypsin in serum during the formation of phagolysosomes. All tumors expressed vimentin, often in a distinctive peripheral cytoplasmic pattern. Focal desmin staining was seen in three separate specimens from the patient with the malignant GCT, but this tumor also expressed S100 protein, myelin basic protein, and Leu-7 and did not stain for muscle-specific actin. The desmin reactivity in this single case probably represents nonspecific staining rather than myogenous differentiation, since the reactivity to other nerve sheath markers shows histogenetic similarity with the benign GCTs. These findings support a Schwann cell origin for nongingival GCTs and illustrate a useful panel of commercially available antibodies to diagnose these distinctive tumors.

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The histogenesis of granular cell tumors (GCTs) has been the subject of extensive immunohistochemical analysis. Many studies have used a limited repertoire of polyclonal antisera or monoclonal antibodies to characterize small series of tumors. Despite some conflicting data, most studies support an origin from Schwann cells. Most GCTs show immunoreactivity for S100 protein, a protein derived from brain that has become a marker for Schwann cells in peripheral nerve tissue.[1-12] S100-protein expression is not limited to Schwann cell neoplasms, however, and a variety of nonneural tumors has been shown to be immunoreactive to anti-S100 antibodies.[6] Some GCTs have expressed other neural markers, including myelin basic protein (MBP),[13] neuron-specific enolase,[8,11,14,15] peripheral nerve myelin proteins,[5] Leu-7,[1] and a melanoma-associated antigen, NK1/C-3, a neural crest marker.[14] Granular cell tumors generally do not express histiocytic, muscle, epithelial, or endothelial-associated antigens.[1,8,9,14]

We analyzed a series of routinely processed GCTs, including one clinically malignant tumor, using a broad spectrum of commercially available antibodies. This panel of antibodies provides additional insight into the histogenesis of these tumors, and also provides a practical tool for differential diagnosis in difficult cases.

MATERIALS AND METHODS

The files of the Division of Surgical Pathology at University Hospital of the University of Alabama at Birmingham were searched for all GCTs diagnosed from 1977 through 1988. We reviewed the slides to confirm the diagnosis of GCT, and then selected a representative block for immunohistochemical analysis. All tissue was fixed in 10% formaldehyde solution and embedded in paraffin.

The tumors were stained with commercially available antibodies using standard peroxidase-antiperoxidase or avidin-biotin-complex techniques (Table 1). Briefly, 5-μm sections were deparaffinized, rehydrated, and immersed in methanol 3% (wt/vol) hydrogen peroxide to quench endogenous peroxidase activity. Slides for α_1-antichymotrypsin (AACT) were trypsinized for 8 minutes at 37°C in a 0.1% (wt/vol) calcium chloride, 0.1% (wt/vol) porcine trypsin solution. Primary antibodies were incubated for 30 minutes at room temperature. The antibodies against neurofilament protein and desmin required an additional 90-minute incubation. Subsequent incubations were performed using either the Vectastain avidin-biotin-complex kit (Vector Laboratories, Burlingame, Calif) or swine anti-rabbit immunoglobulin and rabbit peroxidase-antiperoxidase (Dako Corp, Carpinteria, Calif). The sections were developed using 0.05%

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(wt/vol) 3,3- diaminobenzidine as chromogen and were briefly counterstained with hematoxylin. Tissues known to be reactive were included as positive controls. Two series of negative controls were used. In one, the primary antibody was omitted but the tissue was otherwise tested in a similar manner. For another, nonimmune serum of the same class used at the same dilution was substituted for the primary antibody.

Six GCTs, including the clinically malignant case, were also stained for muscle-specific actin (Enzo Biochem Inc, New York, NY). This monoclonal antibody was used at a 1:8000 dilution and stained using the avidin-biotin-complex technique.

Each of us reviewed the immunoperoxidase-stained slides without knowledge of the clinical setting and graded the staining intensity (0, negative; 1, faint; 2, moderate; 3, strong staining) and the extent of tumor staining (1, ≤50% of cells reactive; 2, 50% to 90% of cells reactive; 3, ≥90% of tumor cells reactive). When there was a difference in interpretation, we reviewed the cases jointly and reached a consensus.

RESULTS

We retrieved 24 specimens from 19 patients for review. All showed histologic features typical of GCTs (Fig 1). The specimens available for study included 17 excisional biopsies, 3 endoscopic biopsies, 1 needle biopsy, and 3 wide excisions. The patients ranged in age from 18 to 73 years and included 17 women and 2 men. Tumor sites were vulva (6), superficial soft tissues (5), skin (4), tracheobronchial tree (3), skeletal muscle (1), tongue (1), hypopharynx (1), esophagus (1), rectum (1), and anus (1). One patient had three separate benign tumors involving skeletal muscle (1), vulva (1), and skin (1), which were excised over a 4-year interval. One additional patient had a regional soft-tissue recurrence and pulmonary metastases 3 years after wide excision of a large primary skin tumor, and she died of widely disseminated disease 4 years after diagnosis. All three specimens from this individual showed moderate nuclear pleomorphism and a focally high mitotic rate (Fig 1). Electron microscopy of this case showed features characteristic of a GCT, including many complex phagolysosomes. There was no ultrastructural evidence of muscle differentiation in the malignant GCT.

Table 1.—Summary of Antibodies Used*

Antigen	Clonality	Dilution	Method
S100	Polyclonal†	1:300	PAP
Leu-7	Monoclonal‡	1:10	ABC
MBP	Monoclonal§	Prediluted	ABC
GFAP	Polyclonal†	1:300	PAP
Vimentin	Monoclonal†	1:10	ABC
Desmin	Monoclonal§	1:30	ABC
NF	Monoclonal§	Prediluted	ABC
Cathepsin B	Monoclonal||	1:150	ABC
AACT	Polyclonal†	1:400	PAP

*S100 indicates S100 protein; PAP, peroxidase-antiperoxidase; ABC, avidin-biotin-complex; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; NF, neurofilament protein; and AACT, α_1-antichymotrypsin.

†Dako Corp, Carpinteria, Calif.

‡Becton-Dickinson, Mountain View, Calif.

§Biogenex, San Ramon, Calif.

||ICN ImmunoBiologicals, Lisle, Ill.

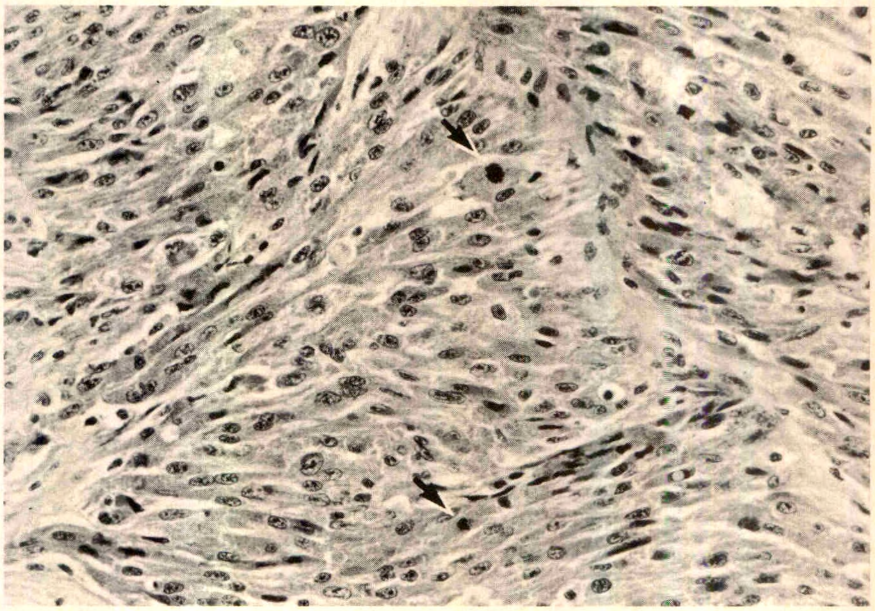

Fig 1.—Malignant granular cell tumor composed of moderately uniform cells with abundant granular cytoplasm. Note mitotic figures (arrows) (hematoxylin-eosin, ×260).

Table 2.—Immunohistochemical Results: 22 Granular Cell Tumors*

Antigen	No. Positive (%)	Mean Staining Intensity (Range)	Mean Extent of Staining (Range)
S100	22 (100)	3.0 (. . .)	3.0 (2-3)
Leu-7	13 (59)	1.4 (1-2)	1.1 (1-2)
MBP	8 (36)	1.3 (1-2)	1.0 (. . .)
GFAP†	0	. . .	. . .
Vimentin	22 (100)	1.9 (1-3)	2.2 (1-3)
Desmin	1 (5)	1.3 (1.2)	1.0 (. . .)
NF	0	. . .	. . .
Cathepsin B	21 (95)	1.9 (1-3)	2.3 (1-3)
AACT	15 (68)	1.7 (1-3)	2.0 (1-3)

*S100 indicates S100 protein; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; NF, neurofilament protein; and AACT, α_1 antichymotrypsin.

†Glial fibrillary acidic protein staining performed on 23 of 24 cases.

Table 2 summarizes the results of immunoperoxidase staining. All cases showed diffuse, strong cytoplasmic, and nuclear staining for S100 protein (Fig 2). Faint to moderate staining was seen for Leu-7 and MBP in 13 cases and 8 cases, respectively, and was usually focal in distribution (Fig 3). One case stained only for MBP, 7 stained for MBP and Leu-7, and 6 cases stained only for

Leu-7. No cases stained for glial fibrillary acidic protein, and none of our cases stained with the antineurofilament antibody.

All cases stained for vimentin. The staining was usually moderate in intensity, and the brown reaction product often decorated the cytoplasmic membranes in a distinctive peripheral pattern (Fig 4). All three specimens from the patient with the clinically malignant tumor showed focal cytoplasmic staining for desmin (Fig 5). These three specimens also stained for S100 and Leu-7 and showed the strongest staining for MBP. There was no muscle-specific actin reactivity in the six cases studied, including the malignant GCT that showed focal desmin staining.

Cathepsin B showed diffuse cytoplasmic positivity in all but a single case. The staining was generally moderate in intensity and accompanied by background staining. Staining for AACT was seen in 15 cases and tended to be moderate in intensity. The staining was diffuse in most instances, although only focal staining was seen in five cases. Two cases showed a distinct perinuclear staining pattern for AACT.

COMMENT

Despite numerous attempts to establish the histogenesis of GCTs, conflicting data still exist regarding the most probable cell of origin. Our results agree with studies showing S100 protein expression in most nongingival GCTs. A notable exception is the study of Buley et al[14] in which only 3 of 25 cases showed a positive immunoreaction to a monoclonal S100 antibody S1-61. The reason for this discrepancy is uncertain, although this monoclonal antibody shows more limited reactivity with neural tumors. A few other reported GCTs have failed to show reactivity for S100 protein. For instance, Ulrich et al[11] found that S100 protein stained 14 of 18 tumors, with tumors located in the brain, pituitary, and thyroid nonreactive. Nathrath and Remberger[8] found S100 protein staining in eight of nine GCTs; the single exception was a subcutaneous tumor overlying the clavicle. S100-protein expression in the majority of nongingival GCTs, including all those in our study, strongly support the origin of these tumors from Schwann cells of peripheral nerves.[1-10,12] The absence of S100 staining in GCTs of the central nervous system, gingiva, and occasional tumors of soft tissue containing cells with granular cytoplasm suggests a different cell of origin for these tumors.

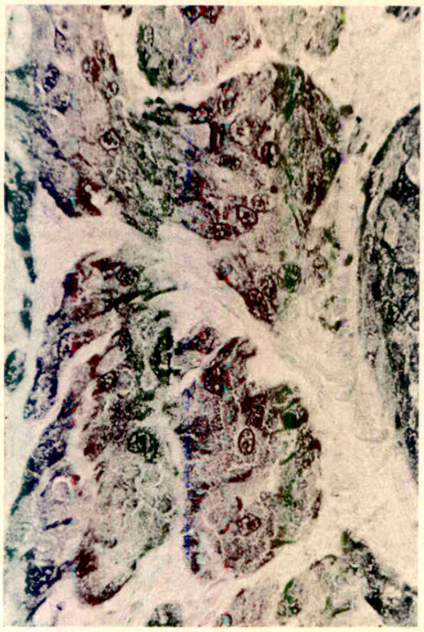
Fig 2.—Intense, diffuse S100 immunoreactivity in a granular cell tumor (hematoxylin counterstain, ×260).

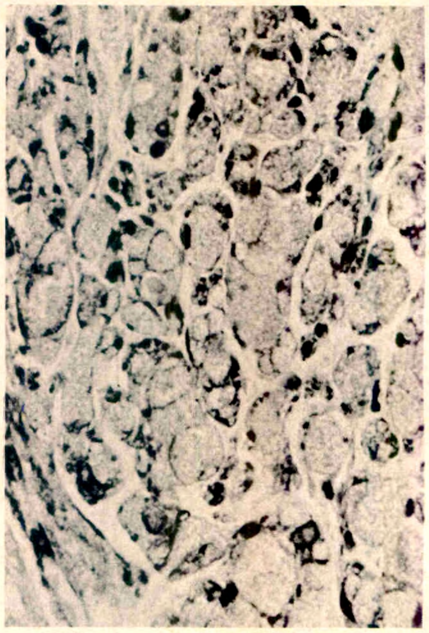
Fig 4.—Staining for vimentin is largely restricted to the peripheral cytoplasm (hematoxylin counterstain, ×496).

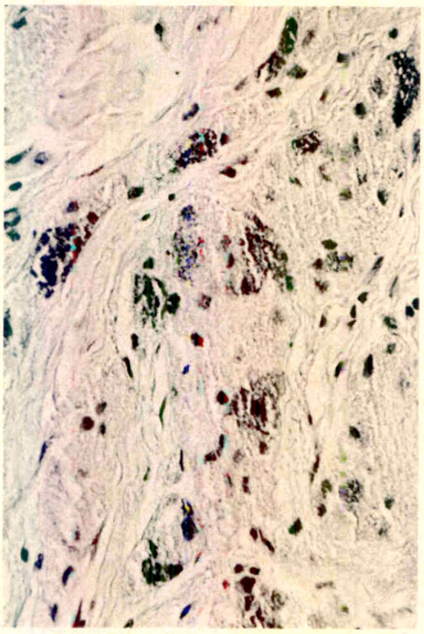
Fig 3.—Patchy staining for Leu-7 in a soft-tissue granular cell tumor of the abdominal wall (hematoxylin counterstain, ×260).

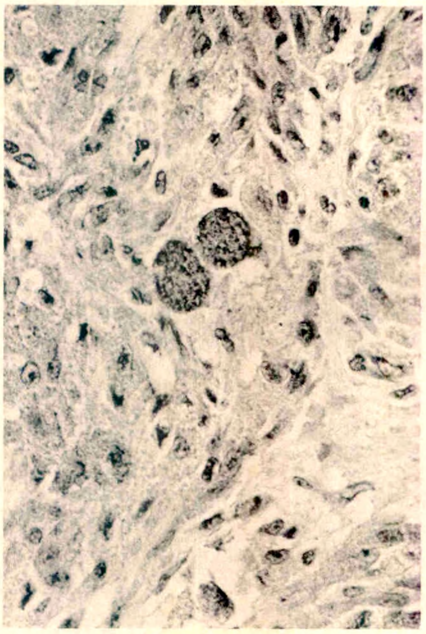
Fig 5.—Several cells in the malignant granular cell tumor show cytoplasmic reactivity for desmin (hematoxylin counterstain, ×260).

Other neural markers have also been identified in GCTs. Myelin basic protein, a component of the central nervous system and peripheral nerve myelin, was present in over one third of our tumors. Penneys et al[13] also showed MBP in GCTs, although they gave neither the number of reactive tumors nor the extent of reactivity. Mukai[5] showed the presence of P2 protein and P0 protein, two distinct peripheral myelin proteins, as well as S100 protein in 18 cases of GCT. In contrast, Clark et al[3] were unable to find MBP or expression of P2 protein in the six cases they studied. Leu-7 is a monoclonal antibody that recognizes myelin-associated glycoproteins and has shown immunoreactivity with central and peripheral nervous system tumors. Leu-7 staining is not specific, however, and is present in a wide variety of neuroendocrine tumors.[16] We found focal staining for Leu-7 in over half of our cases. Leu-7 was usually coexpressed with MBP. Abenoza and Sibley[1] also found Leu-7 staining in two cases of GCT. The presence of MBP and Leu-7 in the majority of our GCTs lends additional support to a Schwann cell origin for these tumors.

Several other immunohistochemical studies of GCTs have analyzed for the presence of AACT and other "histiocyte" markers, reporting negative results in most GCTs studied.[8-12,14,17,18] Our results, in contrast, show frequent immunoreactivity of GCTs to AACT and to cathepsin B. The reason for this apparent discrepancy in our data compared with previous studies is not known to us. Our tests of both positive and negative controls indicated that our antibodies were reacting appropriately. Although histiocytes contain AACT and cathepsin B, both antigens have a broad tissue distribution and are not specific markers for a particular cell type.[18-20] Cathepsin B is a lysosomal proteolytic enzyme, and AACT belongs to the plasma protease-antiprotease system with a putative function to inactivate the proteolytic enzyme cathepsin G. The presence of both these substances in GCTs is probably related to the formation of the many phagolysosomes in these tumors. These cells may either produce AACT during the phagocytic process[19] or AACT may become incorporated from serum into the cytoplasm during phagocytosis.[21]

Although background staining was high, we believe the GCTs do show reactivity for cathepsin B since all controls were satisfactory for the procedure. The presence of cathepsin B in GCTs may be related more specifically to lysosomal digestion of myelin. Cathepsin B is an enzyme that digests myelin basic protein and β-lipoprotein. Normal peripheral nerves do not contain cathepsin B,[20] but this enzyme may play an important role in certain demyelinating diseases.[22,23] Other studies have shown that the cytoplasmic granules of the GCT contain in part myelin that has undergone autophagocytosis.[5,24] Our data suggest that cathepsin B may participate in autodigestion of those proteins.

Intermediate filaments represent an important group of cytoplasmic antigens that have utility in classification of tumors. Previous studies have found that most GCTs express only vimentin,[11,14,18] while keratins, desmin, actin, and neurofilament have not been present.[1,4,8-10,11,14,18] Vimentin staining was present in all our tumors and usually had a distinctive peripheral cytoplasmic staining pattern. This staining pattern is illustrated by others[18] and appears to be a characteristic feature of GCTs. We know of no ultrastructural findings to explain the peripheral distribution of vimentin in these tumor cells.

The absence of desmin and actin and negative immunostaining for myoglobin and myosin[5,8,10,11,14] militate against a myogenous origin for the GCT. Unexpectedly, immunostaining for desmin was seen in a minority of tumor cells in all three specimens from the same patient with the malignant GCT. The consistent presence in all specimens from this tumor makes it unlikely that this result was a staining artifact. This patient had a metastasizing tumor that showed focal nuclear pleomorphism and a high mitotic rate. Electron microscopy showed cytoplasmic granules typical of GCTs, but we found no cytoplasmic filaments to explain the desmin expression. The reason for desmin reactivity in this unusual neoplasm is not known. This tumor showed strong reactivity for S100 protein, MBP, and Leu-7, so we believe its histogenesis is similar to that of the other GCTs in this study. Previous reports of malignant GCTs are rare, and few have been studied by immunohistochemistry. Khansur et al[25] observed S100 staining in two malignant GCTs, and Troncoso et al[26] found immunoreactivity for S100 protein and Leu-7 in their single example. No staining was seen for vimentin or desmin in the latter case. Since the tumor in our study did not show reactivity for muscle-specific actin, we doubt that the desmin staining in our case reflects divergent differentiation toward muscle cells. The desmin immunostaining may represent transfer of this protein from muscle cells destroyed by the invading GCT cells. The reactivity may be analogous to the occasional staining of nonmuscular cells for myosin observed when malignant tumors infiltrate skeletal muscle.[27] Myosin immunostaining in such cases may reflect phagocytosis or passive diffusion of myosin into nonmuscular cells, and the desmin immunostaining encountered in the GCT may reflect a similar phenomenon. Regardless, this case illustrates the utility of using a panel of antibodies in establishing the best classification for occasional tumors that show unexpected immunohistochemical results.

The differential diagnosis of GCT is limited and depends to some extent on the affected site. True GCTs arising in soft tissues may occasionally be confused with rhabdomyomas, leiomyomas, hibernomas, fibroxanthomas, and reactive inflammatory processes. The presence of diffuse S100 immunoreactivity along with staining for MBP and Leu-7 should help to distinguish GCT from these entities. Demonstration of coexpression of these neural markers is especially helpful in questionable cases, since none of them alone are immunospecific for nerve sheath tumors.[28,29] Granular cell tumors also may be difficult to distinguish from oxyphilic epithelial tumors in certain sites, particularly in small biopsy specimens. Indeed, a rectal tumor in our series initially was interpreted as a carcinoid tumor. Again the expression of nerve sheath antigens coupled with distinctive staining pattern for vimentin can assist in separating GCTs from other tumors.

In summary, GCTs have a characteristic immunophenotype using commercially available antibodies. In our experience, S100 protein and vimentin are uniformly expressed, and cathepsin B and AACT are seen in the majority of tumors. About two thirds of GCTs stain for either MBP or Leu-7. These results support a Schwann cell origin for peripheral GCTs and show that these GCTs represent a distinct pathologic entity.

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Palisading Granulomas Associated With Lung Cancer

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• A granulomatous response to neoplastic structures was found in three cases of resected small-cell anaplastic carcinoma of the lung. This consisted of almost continuous rims of palisading epithelioid cells surrounding viable, necrotizing, and necrotic tumor nests. None of the patients had received chemotherapy or radiation treatment prior to surgery, and no clinical, microbiological, or histological evidence of tuberculosis, fungal infection, or rheumatoid disease was found. The granulomatous rim seems to be a response to spontaneous tumor decay.

(*Arch Pathol Lab Med.* 1990;114: 697-699)

The coexistence of neoplastic and granulomatous structures provoke questions about their chronologic and causal relations. Tuberculous pulmonary granulomata were at one time suspected to be responsible for the development of "scar cancers,"[1] although the postinfectious origin of these malignancies has not been convincingly documented. In fact, when cancer and tuberculosis coexist in the same lung, they are usually independent and separate.[2] The reverse relation between granulomata and lung cancer, ie, cancer inducing a granulomatous reaction, appears to have occurred in these three cases.

REPORT OF CASES

CASE 1.—This man was a 58-year-old heavy cigarette smoker. A nodule was found in the upper lobe of his right lung on a routine chest roentgenogram. No tumor structures were observed at bronchoscopy and bronchial washings were negative for malignant cells. The upper lobe of the right lung was resected and a 1.0-cm mass was found in its periphery, unrelated to a major bronchus.

Histologic examination showed irregular solid neoplastic islands separated by copious cellular stroma. The nuclear and cytoplasmic features of the tumor cells were characteristic for small-cell anaplastic carcinoma. While much of the stroma was densely cellular, populated with fibroblasts and lymphocytes, most of the tumor islands were surrounded by a light-staining zone consisting of epithelioid cells with characteristic rectangular, occasionally twisted, vesicular nuclei, and pale ill-defined cytoplasm (Figs 1 and 2). Arteries within the tumor were narrowed by intimal proliferation, and tumor nests were present in the thickened intima of the largest artery. Infarct-type tumor necrosis was found in the center of the mass, and a single anthracosilicotic nodule was attached to the periphery of the tumor. Electron microscopy revealed occasional dense core granules in otherwise undifferentiated and scanty tumor cytoplasm. No lymph nodes were available for our examination.

Postoperatively, the patient received three courses of cyclophosphamide, doxorubicin (Adriamycin), and vincristine chemotherapy, followed by three courses of etoposide and cisplatin. Prophylactic brain irradiation (2000 cGy) was given for five treatments. The patient was alive without recurrence 5.5 years after operation.

CASE 2.—This man was a 60-year-old 40-pack-year cigarette smoker. On routine medical examination, a shadow was found in the upper lobe of his left lung. Bronchoscopy and mediastinoscopy were negative. The upper lobe of his left lung was resected and found to contain an ill-demarcated tumor (2.5 × 1.5 × 1.8 cm) centered around a segmental bronchus, and reaching the pleura. The histologic features of the tumor were characteristic of small-cell anaplastic carcinoma, with central geographic necrosis. Several separate peripheral tumor nodules were in various stages of necrobiosis. An almost continuous zone of large palisading epithelioid cells (Fig 3) surrounded the tumor structures, and was especially conspicuous around the peripheral tumor nests. A zone of active fibroplasia with lymphoplasmocytic infiltrates and scattered lymphoid follicles formed the periphery of the tumor mass. In 3 of 10 bronchopulmonary lymph nodes, multiple small metastatic nests were found, surrounded by confluent fields of epithelioid histiocytes displacing the original population of lymphocytes and coniophages. Dense core granules were found in some tumor cells by electron microscopy.

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Postoperatively, this patient received six courses of cyclophosphamide, doxorubicin, and vincristine chemotherapy, supplemented with cranial and mediastinal irradiation (3000 cGy for 10 doses). The patient was alive on March 30, 1989 (4 years after resection), although he is being investigated for a probable bronchial squamous cell carcinoma.

CASE 3.—This woman was a 66-year-old 60-pack-year smoker. A lung mass was discovered during investigation for polyneuritis. Bronchoscopic investigations, including cytologic examination of bronchial washings and brushings, as well as mediastinoscopy, were negative. Three attempts at fine-needle aspiration, always yielded nondiagnostic necrotic material. The upper and middle lobes of the right lung were resected. An extensively necrotic tumor mass measuring 5.5 cm in diameter, reaching the parietal pleura, and not associated with a major bronchus, was found. Viable tumor structures consisted of a few isolated areas at the periphery of the tumor mass, none of them exceeding 5 mm in diameter. The outlines of tumor cells could be recognized in the necrotic areas. Foci of dystrophic calcification, cholesterol clefts, and impregnation of vascular walls and fibrous septa with chromatin dust were found in the necrotic tumor. The whole mass was surrounded with a thin layer of fibrous tissue. The interface between this capsule and necrotic tumor was populated with either foamy macrophages or palisading cells with nuclear morphology ranging from the pale epithelioid cell type to the densely staining fibroblastic variety (Fig 4). Some viable tumor nests were surrounded with continuous zones of epithelioid histiocytes. No metastatic deposits and no granulomata were found in either the intrapulmonary or mediastinal lymph nodes.

The patient returned home in another province after surgery and, according to clinical data, died from recurrent disease 7 months after the operation. An autopsy was not performed.

None of the patients had any symptoms of rheumatoid disease. No acid-fast or fungal organisms were found in appropriately stained sections.

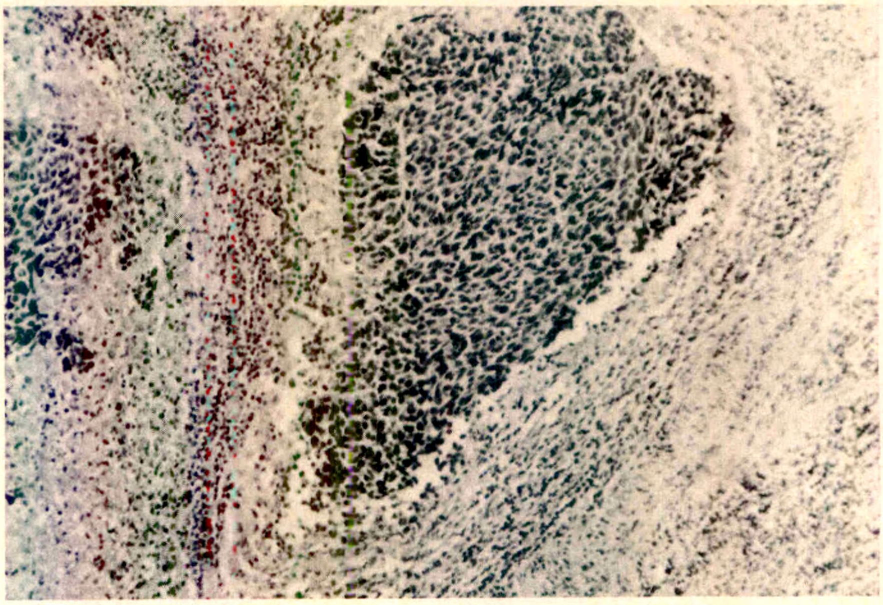

Fig 1.—Patient 1. Nests of small-cell anaplastic carcinoma surrounded with a narrow pale zone of epithelioid histiocytes.

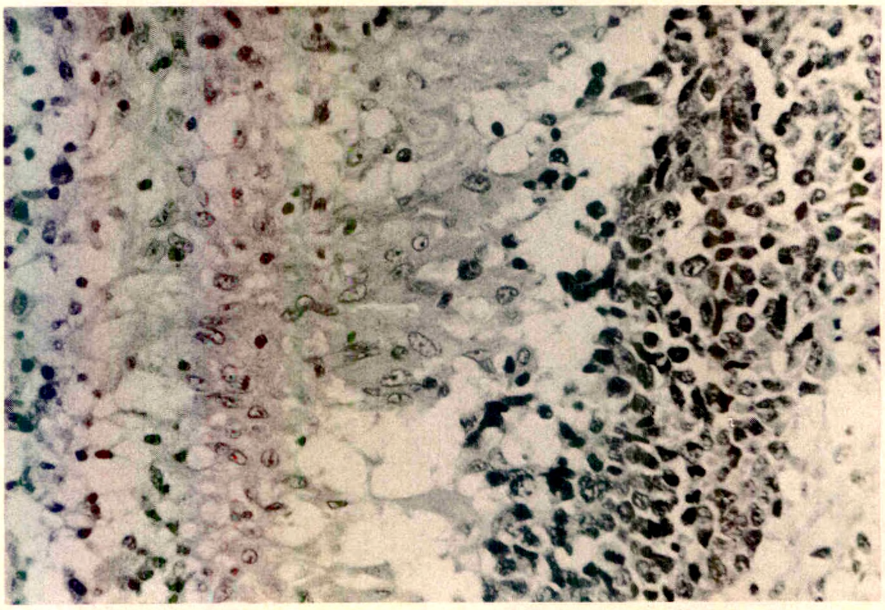

Fig 2.—Patient 1. Detail of epithelioid histiocytes (left center) in contact with carcinomatous nest.

COMMENT

Cancer is listed among conditions capable of inducing a granulomatous tissue response,[3] usually as distant sarcoid-type granulomata either in regional lymph nodes for carcinoma[4-6] or in uninvolved organs of patients with lymphoma.[7] In cancer-free lymph nodes such granulomata have been reported in 3.2% of a large series of patients with lung cancer.[8] Except as a foreign body-type reaction to keratin in squamous cell carcinomas, granulomatous response has rarely been found in the tumor proper. Recently, sarcoidlike lesions have been described in the stroma located entirely within tumor tissue of three invasive breast carcinomas.[9]

In our patients, the granulomatous reaction was formed by palisading rows of epithelioid cells, similar to those in rheumatoid granulomas or at the edges of caseation, which surrounded live or necrotic tumor structures. None of our patients had any clinical or pathologic evidence of rheumatoid arthritis, tuberculosis, or fungal infection, and shadows of undoubted cancer cells could be recognized in necrotic areas. The epithelioid cell palisades around the tumor structures then most likely represent a reaction elicited by substances released from decaying tumor cells. Similar pali-

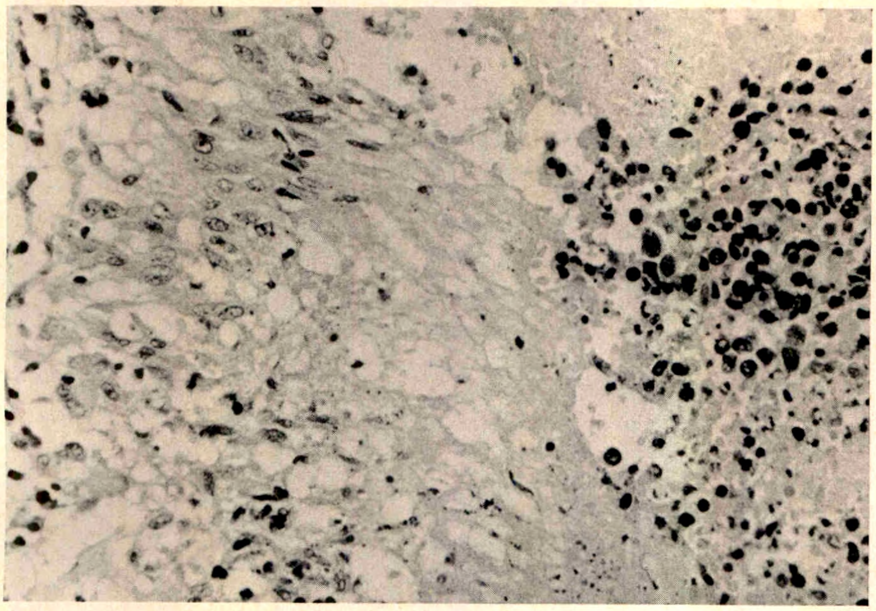

Fig 3.—Patient 2. Epithelioid histiocytes (left) forming a rim around necrotizing carcinoma.

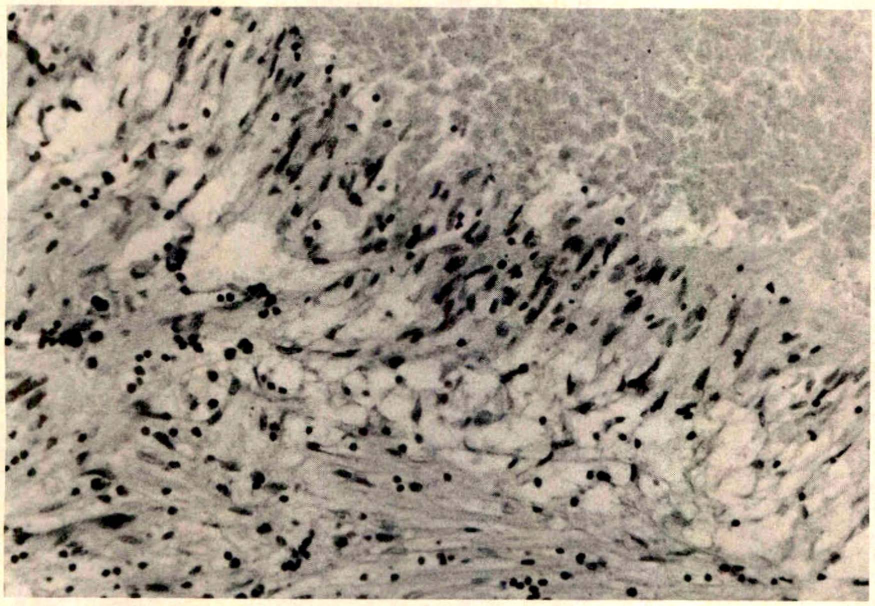

Fig 4.—Patient 3. Palisading histiocytes at the edge of necrotic carcinoma. Shadows of necrotic tumor cells are still recognizable.

sading granulomas have been described in the prostate, kidney, cervix, and fallopian tube,[10-13] always antedated by a surgical procedure, such as needle biopsy or curettage. Damaged or necrotic tissue was apparently the stimulus provoking histiocytic demarcation. Although our cases lacked iatrogenic tissue damage (patients 1 and 2) and the epithelioid rim was not related to needle tracks (patient 3), the pathogenetic mechanism reaction to substances released from damaged tissue appears to be similar.

While small-cell cancers represent a minority among resected lung cancers (7 of 245 in our material), they seem to be the only ones to induce palisading granulomas. Two of our patients were alive without recurrence 4 years after treatment, exceeding the mean survival time for this type of cancer treated by chemotherapy.[14] In the other patient, who died within months of resection, the epithelioid palisades were discontinuous. Whether the granulomatous reaction itself has a protective role or is a marker of some other effective antitumor defense, will remain hypothetical until larger numbers of similar cases can be assembled for analysis.

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Carcinoid Tumors of the Duodenum

A Clinicopathologic Study of 99 Cases

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LTC Kris M. Shekitka, MC, USAF; Elson B. Helwig, MD

• Ninety-nine carcinoid tumors of the duodenum were studied. Seventy-seven patients were followed up for a mean period of 65 months, 20 tumors were autopsy findings, and two patients were unavailable for follow-up. Sixteen tumors (21%) produced metastases, all discovered initially; 3 patients (4%) died from metastatic disease (mean survival, 37 months postoperatively). Features associated with metastatic risk were involvement of muscularis propria, size greater than 2 cm, and the presence of mitotic figures. For 51 tumors, there was no correlation between immunohistochemical somatostatin and history of diarrhea, cholelithiasis, or diabetes mellitus (somatostatin syndrome). Five tumors were associated with Zollinger-Ellison syndrome and had immunohistochemical gastrin, but in the others there was no correlation between ulcer disease and gastrin positivity. Duodenal carcinoids are indolent, especially when small and localized to the submucosa. Immunohistochemical identification of somatostatin and gastrin has little clinical relevance.

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Carcinoids that arise in the duodenum are rare, and for this reason are sometimes discussed with other small-bowel carcinoids.[1,2] Unlike carcinoids of the jejunum and ileum, duodenal carcinoids are often discovered endoscopically. Little follow-up information is available on patients with duodenal carcinoids,[3-5] especially those who have had endoscopic biopsy only. The purpose of this study is to determine which pathologic parameters are predictors of metastasis, to help direct management of patients with duodenal carcinoids, including those with endoscopically biopsied tumors.

Duodenal carcinoids differ further from carcinoids of the jejunum and ileum in that they are associated with Zollinger-Ellison syndrome (ZE),[3,4,6] often demonstrate immunohistochemical somatostatin,[5] and rarely cause the carcinoid syndrome.[3-5,7] An additional goal of this study was to compare the presence of immunohistochemical gastrin and somatostatin with clinical signs and symptoms of gastrin and somatostatin secretion to determine if immunohistochemical stains are clinically relevant.

MATERIALS AND METHODS

Between 1970 and 1986, 110 carcinoids of the duodenum were accessioned to the Armed Forces Institute of Pathology, Washington, DC. The diagnosis was based on light-microscopic World Health Organization, Geneva, Switzerland, histologic criteria.[7,8] We have recently reported the immunohistochemical findings of 65 of these tumors and have shown that they have a distinct immunohistochemical profile.[7] We have subsequently acquired detailed clinical and/or follow-up data on 51 of the previously presented patients and an additional 44 cases. Our previous article[7] tabulated positive immunohistochemical reactions to determine specificity of endocrine markers for these tumors and to correlate psammoma bodies with somatostatin positivity and von Recklinghausen disease (vRD). In contrast, this article presents follow-up data, and describes immunohistochemical data only as they relate to clinical syndrome associated with overproduction of gastrin and somatostatin. These hormones were demonstrated immunohistochemically in a large number of cases.[7]

Follow-up consisted of hospital records, questionnaires (with specific emphasis on preoperative symptoms of peptic ulcer disease, diarrhea, cholelithiasis, and diabetes mellitus), and Tumor Board requests. Cases were included for study if adequate clinical information was available. This was defined as an operative report and results of abdominal computed tomography and liver spleen scans to assess the presence of metastases, a minimum of 2-year follow-up, or autopsy protocol.

Immunohistochemical stains were performed by standard techniques[7,9] on 51 tumors for which paraffin-embedded blocks were available. Polyclonal antisera against somatostatin (neat, purified) and N-gastrin (1:400 dilution) were obtained from IncStar Corp (San Diego, Calif). All stains were performed with appropriate positive and negative controls run in parallel. Positive control for somatostatin was normal pancreas; control for N-gastrin was a section of normal duodenum. The N-gastrin antibody was directed against the 16-amino acid fragment of 34 gastrin (big gastrin), and shows no cross-reactivity with the C-terminal gastrin-17, little gastrin (communication from technical staff, IncStar Corp). Specificities of all reagents were determined by the manufacturers. Mitotic counts were performed on 5-μm sections using a 40-power objective (490-μm-field diameter).

Seventy-two cases were sent from civilian, 16 from military, and 11 from Veteran's Affairs pathologists. Pathologic parameters were compared with the presence of metastatic disease by means of 2 × 2 contingency tables (Fisher's Exact Test).

RESULTS

Clinical and Pathologic Data

Fifty-nine patients were male and 40 were female. The age range was 33 to 90

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years (mean, 59 years). The presenting symptom or sign was abdominal pain (34), upper gastrointestinal tract bleeding (11), jaundice (7), anemia (5), lower gastrointestinal tract bleeding (4), vomiting (4), gastritis (3), duodenal obstruction (1), and diarrhea (1). Twenty tumors were found initially at autopsy: in 1, a large tumor contributed to the cause of death by ulcerating and bleeding; in the remainder, the carcinoid was incidental. Six tumors were discovered at cholecystectomy, and 4 were detected during workup for other malignancies (gastric carcinoma, esophageal squamous carcinoma, colon carcinoma). Two were identified during a familial workup; these patients and the proband have been published separately.[10] The size of the tumor was known in 96 cases, ranged from 0.2 to 5.0 cm, and averaged 1.8 cm. Thirty-four tumors were located in the first part, 41 in the second part, 2 in the third part, and 2 in the fourth part of the duodenum. In the remaining 20 tumors, exact location in the duodenum was not recorded. Thirteen tumors were multiple, 5 in women and 8 in men. The histologic pattern was usually a mixture of glandular, solid, insular, and occasionally trabecular structures. Fifteen tumors contained a number of psammoma bodies; 14 of these were in the second part of the duodenum, and were largely glandular. Fifteen tumors in the second part of the duodenum were specifically located in the ampulla. Of these, 6 patients had vRD and 7 tumors had psammoma bodies. Ninety-one tumors had fewer than one mitosis per 10 high-power fields; 8 had mitotic rates of between one and three per 10 high-power fields. One tumor had small areas of necrosis. For 79 tumors depth of invasion could be assessed on the basis of the histologic material available for study. Thirty-nine were confined to the submucosa (Fig 1), and 40 involved the muscularis propria (Fig 2). Of 51 tumors studied immunohistochemically, 25 were somatostatin positive, and 26 were gastrin positive. Three tumors were positive for both somatostatin and gastrin.

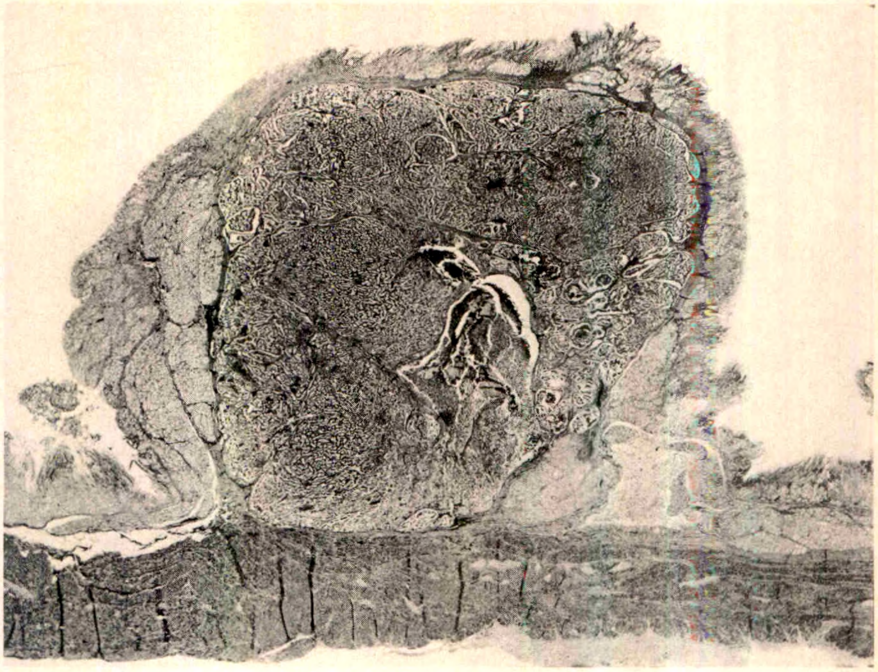

Fig 1.—Duodenal carcinoid tumor. Circumscribed 2-cm tumor that does not infiltrate muscularis propria (× 4).

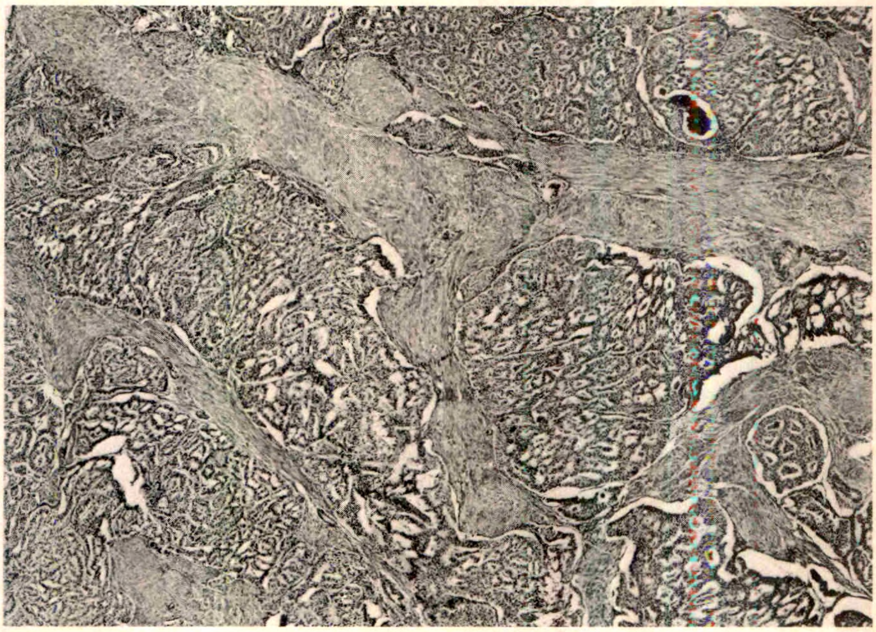

Fig 2.—Duodenal carcinoid tumor. Tumor infiltrates muscularis propria (× 150).

Follow-up Data (79 Surgical Patients)

In 41 patients, the initial diagnosis was made by endoscopic biopsy. Of these patients, 12 were followed up without further resection. They were followed up for an average of 37 months. Endoscopically estimated tumor size ranged from 0.4 to 5.0 cm. Of these 12 patients, 11 were alive and well and 1 (with the 5-cm tumor) died at 23 months from alcoholic cirrhosis.

Thirty-eight patients were treated initially by surgery without preoperative biopsy diagnosis, and 29 patients were operated on after endoscopic biopsy. Of these 67 patients, 36 underwent open excision (shelling out of tumor without enteroenterostomy); 31 were treated with sleeve resections, 7 of whom required partial pancreatectomy in addition. No patient with resection developed recurrence at the anastomotic site. There was no evidence of mucosal recurrence in 34 patients with open biopsy; 2 patients with large tumors underwent a biopsy without attempt at excision, and subsequently died of tumor hemorrhage.

Seventy-seven patients were followed up for a mean of 65 months: 45 (58%) were alive and well (mean follow-up, 53 months); 13 (17%) died of other causes (mean follow-up, 60 months); 2 died of postoperative complications (1 after a Whipple's procedure, and 1 after attempted open biopsy), and 1 of tumor hemorrhage (10 months postoperatively). Sixteen patients had metastases (20.8%; mean follow-up, 76 months); of these, 3 died from metastatic disease. All metastases were discovered at initial workup or at initial surgery. Mortality was 5.3% of all patients in the follow-up group, including the additional 1 who died from tumor hemorrhage. Pathologic data, treatment information, and follow-up for those patients with metastases are presented in Table 1.

Table 1.—Duodenal Carcinoids: Patients With Metastases*

Involvement of Muscularis Propria	Size, cm	Mitoses/ 10 hpf	No.	Metastases	Months Follow-up	Outcome Treatment
Absent	2.0	0	1	Lymph nodes	77	NED/resection
Present	3.0	0	1	Lymph nodes	13†	NED/resection
Present	4.5	0	1	Mesentery	359	NED/biopsy
Present	2.0	0	1	Lymph nodes	22	NED/biopsy
Present	4.0	2	1	Lymph nodes	28	NED/resection
Present	1.0	0	1	Lymph nodes	6	NED/resection
Present	3.0	0	1	Liver, lymph nodes	99†	DOC/biopsy
Present	3.0	1	1	Lymph nodes	1	DOC/resection
Present	2.0	0	1	Lymph nodes	63	DOC, NED/biopsy
Present	3.0	0	1	Lymph nodes	2	DOC, NED/resection
Present	3.0	0	3	Liver, lymph nodes	49	AWD/biopsy
Present	2.0	1	1	Liver	185‡	AWD/resection, acid suppressors
Present	1.5	0	2	Lymph nodes	53‡	AWD/resection, acid suppressors
Present	1.3	1	1	Liver	22	DOD/biopsy
Present	2.0	4	1	Liver, lymph nodes§	36	DOD/5-fluorouracil, streptozotocin, RT, dacarbazine, resection
Present	3.0	0	1	Liver, lymph nodes, lung	52	DOD/dacarbazine, doxorubicin (Adriamycin), streptozotocin, 5-fluorouracil biopsy

*The mean age was 53 years; 8 patients were male and 8 patients were female; hpf indicates high-power field; RT, radiation therapy; NED, no evidence of carcinoid; DOD, dead of carcinoid; DOC, dead other causes; and AWD, alive with carcinoid.

†Von Recklinghausen disease.

‡Zollinger-Ellison syndrome.

§Supraclavicular lymph nodes; other lymph nodes were regional.

Table 2.—Duodenal Carcinoids: Risk Factors for Metastasis*

	Metastasis	No Metastasis	*P*
Confined to submucosa	1	38	.00017
Involvement of MP	14	26	
Smaller or equal to 2 cm†	3	51	.001
Larger than 2 cm	13	29	
No mitosis	11	80	.0025
Mitoses	5	3	

*MP indicates muscularis propria. Size of tumor was known for 96 tumors; level of invasion was known in 79 tumors; and mitotic rate was measured for all 99 tumors.

†For a cutoff of 1 or 1.5 cm, the *P* values were .006 and .005, respectively.

Correlation of Clinicopathologic Parameters With Outcome

The presence of metastases was compared with age, tumor size, mitotic activity, and depth of invasion for all patients as well as a subset with at least 24 months follow-up (49 patients). Invasion into the muscularis propria, size, and mitotic activity correlated with metastatic spread; the first carried the strongest statistical significance (Table 2). The average age at time of presentation of patients with metastatic disease was 52 years; the average age of patients without metastatic disease was 60 years.

No tumor smaller than 1 cm metastasized; only one tumor confined to the submucosa metastasized to regional lymph nodes. Eight of 24 tumors larger than 2 cm metastasized, as did 14 of 40 tumors invading the muscularis propria.

Associated Tumors and Conditions

Twenty-eight patients had malignancies other than their carcinoids (Table 3). Seven patients had vRD; all of these tumors were periampullary and somatostatin positive, and 5 had psammoma bodies. Five patients had elevated serum gastrin levels and recurrent peptic ulcers (ZE); the 5 tumors from these patients had only scattered gastrin positive cells (Fig 3). Two tumors were discovered initially at autopsy, were not grossly evident at prosection, and did not have evidence of metastatic spread. These 2 patients died of a gunshot wound and postoperative complications for ulcer surgery, respectively. Two additional patients with ZE syndrome had metastatic disease (Table 1); the fifth patient was alive and free from metastases 191 months after resection of her tumor. Fourteen additional patients had preoperative peptic ulcers without documented hypergastrinemia. Excluding patients with ZE syndrome, there was no correlation between a preoperative history of ulcer disease and immunohistochemical gastrin (Table 4). The extent and strength of gastrin staining was no different in tumors from patients with ZE syndrome or ulcer disease compared with those in patients without such a history.

Results of serum or urine 5 hydroxyindolacetic acid measurements were available in 23 patients. One had a borderline urinary value without definite carcinoid syndrome clinically; the others had normal values. One patient had severe watery diarrhea preoperatively; one had a possible history of flushing, but serum 5 hydroxyindolacetic acid levels were not elevated.

Table 3.—Duodenal Carcinoids Associated Tumors and Conditions

Epithelial tumors	
Adenocarcinoma	
Colorectum	4
Stomach	1
Lung	1
Prostate	4 (3 metastatic)
Squamous carcinoma	
Lung	2
Esophagus	2
Cervix (metastatic)	1
Oral mucosa	2
Colorectal adenomas	3
Oat cell carcinoma, lung	2
Multiple gastric hyperplastic polyps	4
Endocrine proliferations	
Parathyroid hyperplasia	1
Metastatic pheochromocytoma	1*
Adrenal cortical hyperplasia	3
Nodular goiter	3
Ileal carcinoid	1
Rectal carcinoid	1
Pulmonary carcinoid	1
Zollinger-Ellison synrome	5
Von Recklinghausen disease	7
Neurofibrosarcoma, metastatic	1
Prostatic sarcoma	1
Other	
Astrocytoma	1
Malignant lymphoma, FCC type	1
Peptic ulcers (no proof of Zollinger-Ellison syndrome)	21
Hepatic angiomas	2
Meningioma	1
Gastric leiomyoma	1
Cystic thymoma	1
Uterine leiomyoma	1

*This patient had no evidence of neurofibromas or café au lait spots.

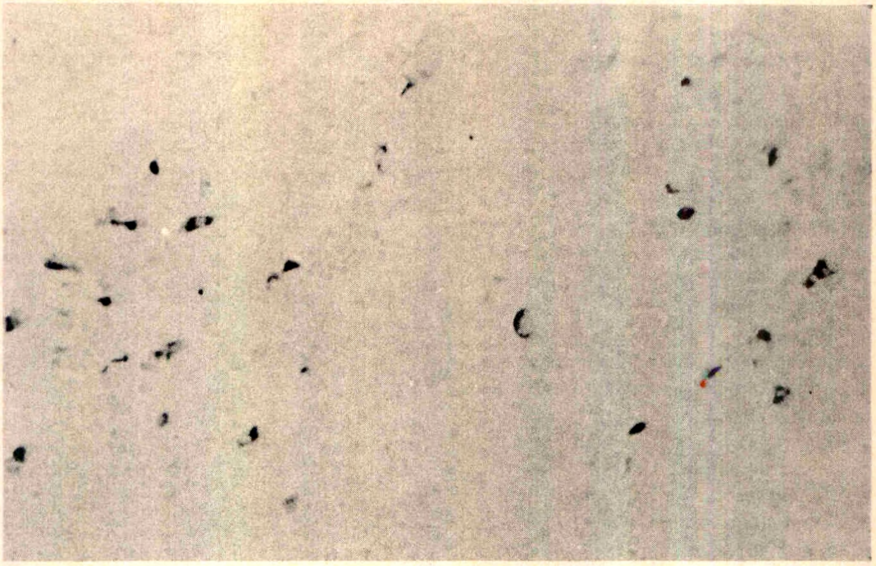

Fig 3.—Duodenal carcinoid tumor. Gastrin present in scattered tumor cells (peroxidase-antiperoxidase, ×150).

Table 4.—Duodenal Carcinoids: Correlation of Immunohistochemical Gastrin and Peptic Ulcer Disease*

	Gastrin Positive	Gastrin Negative
Peptic ulcer disease	8	5
No peptic ulcer disease	13	19

*Patients with Zollinger-Ellison syndrome were excluded.

Table 5.—Correlation of Immunohistochemical Somatostatin and History of Diabetes, Cholelithiasis, and Diarrhea

	Somatostatin Positive	Somatostatin Negative
Diabetes mellitus	1	6
No diabetes mellitus	24	20
Cholelithiasis	6	4
No cholelithiasis	19	22
History of diarrhea	0	2
No history of diarrhea	25	24

Thirteen patients had diabetes mellitus; for 2, it was attributed to postoperative pancreatic insufficiency. Fifteen patients had cholelithiasis at the time of surgery; 5 had had cholecystectomies for stones more than 5 years previously. In 4 patients the admitting diagnosis included diarrhea. There was no correlation between somatostatin positivity and the components of the somatostatin syndrome: diabetes, cholelithiasis, or diarrhea (Table 5).

COMMENT

Because the behavior of gastrointestinal carcinoid tumors is unpredictable, some investigators have advocated surgical resection for all, including those of the small bowel.[3,11] The use of endoscopy has led to the increased diagnosis of incidental duodenal carcinoids, many of which are difficult to resect surgically because of proximity to the ampulla. Prognostic features associated with metastasis have been identified for gastrointestinal carcinoid tumors, and include size and infiltration of the muscularis propria.[12-15] We have shown that although rectal carcinoids metastasize less frequently than those of the duodenum, size, mitotic rate, and depth of invasion are reliable signs of metastatic potential.[16] In this study, we have demonstrated that these features are significantly associated with metastatic disease for duodenal carcinoids as well. Indeed, only one tumor without involvement of the muscularis propria, and none smaller than 1 cm metastasized.

Our follow-up data have important implications for endoscopically diagnosed tumors and those not amenable to surgery. All of our patients who had endoscopy without further surgery have not suffered ill effects from their disease. We believe that if the patient's condition and location of tumor preclude surgical removal, endoscopic treatment of duodenal carcinoids that are under 1 cm in size or confined to the submucosa can be acceptable, especially if mitoses are absent.

The association between duodenal carcinoids and vRD has been amply documented.[5,17] Carcinoid tumors in these patients are ampullary in location, often have psammoma bodies, and contain somatostatin immunohistochemically.[5,7,17,18] There is no ready explanation why patients with vRD develop carcinoid tumors almost exclusively in the ampulla, nor for the high frequency of vRD in patients with duodenal carcinoid tumors. We have found that 7% of patients with duodenal carcinoids have vRD, and nearly 50% of patients with ampullary carcinoids have vRD. Therefore, in patients with ampullary carcinoid tumors, vRD should be clinically ruled out; conversely, if a patient with vRD develops jaundice, an ampullary carcinoid tumor should be strongly considered.[19] Pheochromocytomas have also been described in patients with duodenal carcinoids and neurofibromatosis[20-22]; the single pheochromocytoma in our series was in a patient without neurofibromas. This patient may have a forme fruste of vRD.

Although most patients with ZE syndrome have islet cell tumors of the pancreas, approximately 10% have duodenal carcinoids.[6] In our series of duodenal carcinoids, 5% had documented ZE syndrome. The total proportion of patients with peptic ulcer disease (18%) was lower in our patients than in two earlier series of duodenal carcinoids,[4,17] in which 8 of 21 and 4 of 12, respectively, had ZE syndrome. This may be due to the increased use of endoscopy, resulting in the discovery of more incidental tumors. We were unable to show a correlation between the presence or strength of immunohistochemical gastrin and a history of ulcers; this may in part reflect the antiserum used to detect gastrin in the tumor tissue. We employed the N-gastrin antiserum that detects big gastrin, which is biologically less active than gastrin-17.[23] Another possible explanation for the lack of correlation between symptoms and immunohistochemical findings is that tumors can excrete high levels of hormone with little cytoplasmic storage.[24] The limitations of immunohistochemistry in evaluating hormonal production may eventually be overcome by concomitant use of RNA in situ hybridization, which has the potential for determining polypeptide synthesis.

The somatostatinoma syndrome (diabetes mellitus, diarrhea, cholelithiasis) can be a manifestation of pancreatic islet cell tumors,[25] but it is not an obvious feature of somatostatin containing duodenal carcinoids. None of the patients in our series presented with the classic triad. Somatostatin serum levels were not drawn on any of the patients. We compared immunohistochemical somatostatin with individual symptoms of the syndrome and found no correlation. Immunohistochemical stains do not necessarily reflect serum levels of polypeptides; however, we believe that these data are evidence that somatostatin production in duodenal carcinoids is rarely clinically relevant.

A number of malignancies other than duodenal carcinoids were diagnosed in our patients (Table 3); three had other carcinoids (ileum, lung, rectum). The high frequency of endocrine and gastrointestinal neoplasia may be a result of increased surveillance in our patients, who were, for the most part, extensively investigated.

We have documented the relatively indolent nature of duodenal carcinoids, and have shown that spread into the muscularis propria, a size greater than 2 cm, and mitotic activity are significant risk factors for metastasis. Many studies have emphasized the association with vRD or ZE syndrome, but only a minority of patients with duodenal carcinoids appears to have either condition. Although gastrin and somatostatin are frequently demonstrated in duodenal carcinoids, this is apparently of little clinical relevance.

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Fine-Needle Aspiration Cytology of Malignant Hemangiopericytomas With Ultrastructural and Flow Cytometric Analyses

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• A hemangiopericytoma (HPC) is an uncommon soft-tissue neoplasm that may arise in many body sites. The cytologic features of fine-needle aspirates (FNAs) of HPCs have only rarely been described in the literature. We examined FNAs of malignant HPCs from the head and neck region (three) and the retroperitoneum (one) in four adults (aged 38 to 83 years). All four FNAs yielded cellular specimens that consisted of uninuclear tumor cells with high nuclear-cytoplasmic ratios. The cytomorphological spectrum included nuclei that were oval to elongate and had very finely granular, evenly distributed chromatin with one or two small but distinct nucleoli. Hemangiopericytomas yield aspirates that may be considered malignant and may suggest sarcoma. Histologically, all four neoplasms manifested high mitotic activity. The ultrastructural features of all four tumors were supportive of the diagnosis of HPC. Although a specific primary diagnosis of HPC on FNA of a soft-tissue mass is unlikely, cytologic analysis may allow diagnosis of recurrent or metastatic HPC. We were able to perform flow cytometric determinations of tumor DNA content on three of the resected neoplasms. In two, an aneuploid pattern was found, including the neoplasm with the most marked pleomorphism in the FNA. The third was diploid.

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As a diagnostic technique, fine-needle aspiration (FNA) has become accepted by many physicians and continues to enjoy growing popularity. Yet its application in the primary diagnosis of soft-tissue lesions has seen only limited utility.[1-6] In part, this is due to the relative infrequency of clinically significant soft-tissue tumors and to the difficulty in diagnosing certain soft-tissue lesions, even with histologic examination. This includes both differentiation of benign from malignant mesenchymal proliferations and determination of the exact histogenesis or cell type.

Malignant hemangiopericytomas (HPCs) are uncommon soft-tissue sarcomas that generally occur in adult patients as mass lesions without specific clinical or radiographic features.[7-10] They arise most often in the lower extremities, the pelvis, and the retroperitoneum, but also occur with some frequency in the head and neck region. The individual tumor cells of HPC do not have distinguishing morphological features on either histologic examination or immunohistochemical analysis. Ultrastructural examination often lends a degree of specificity to this diagnosis.[11-13] Histologic criteria predicting a malignant biologic potential include brisk mitotic activity, cellular pleomorphism, and tumor necrosis.[7-10]

Only rare cytomorphological descriptions of HPC in FNA specimens exist.[14,15] We present the aspiration cytologic features of four malignant HPCs, all of which had ultrastructural confirmation. In addition, we report the DNA content of the neoplastic cells; we are unaware of any such similar report.

PATIENTS AND METHODS

The clinical features of the patients are summarized in Table 1. In patients 2 through 4, the FNAs were obtained from superficial masses using a 21- or 22-gauge needle. In patient 1, FNA was performed under computed tomographic guidance. In all cases, direct smears of the aspirated material were made by routine methods. Approximately one half of the slides were air-dried and stained promptly with Diff-Quik. The other

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slides were immediately fixed in ethanol and stained by the Papanicolaou technique. At least four slides were available in all cases.

Surgically obtained tissue was processed for histologic examination by routine methods. Tumor for transmission electron microscopy was fixed initially in glutaraldehyde and processed routinely.[16]

Nuclear suspensions for flow cytometric analysis were prepared from the three tumors for which adequate tissue was available by a modification of Hedley's procedure.[17,18] Briefly, three consecutive 50-μm sections were obtained from one paraffin-embedded block of each tumor. Also, 5-μm sections bracketing each set of the thicker sections served as histologic controls. The thicker sections from each tumor were placed in a single cassette and deparaffinized using Fisher's Hemo-DE. Subsequently, the sections were rehydrated using a series of graded alcohols (100%, 95%, 70%, and 50%) and washed twice with distilled water. The sections were then finely minced and transferred to a glass test tube to which 1 mL of 0.5% pepsin, titrated to a pH of 1.55 with hydrochloric acid, was added. The specimens were incubated in a 37°C shaker water bath for 45 minutes and subjected to intermittent vortexing. The resulting suspension was washed twice in phosphate-buffered saline. The resulting pellet was stained with 3.4 mmol/L citrate buffer (pH, 7.6) containing 50 μg/mL propidium iodide, 0.6% NP-40, 37 μg/mL ribonuclease, and 0.01 mol/L sodium chloride. The suspension was passed through a 80-μm nylon mesh prior to analysis.

Analysis of DNA content was performed using a flow cytometer (FACS Analyzer, Becton-Dickinson, Mountain View, Calif) that employs a mercury arc lamp as its excitation source. Excitation occurred over the range of 474 to 496 nm. The emitted light was analyzed for forward and right angle light scatter and red fluorescence (565 to 625 nm). The samples were not gated and 20 000 events were analyzed. Lymphocytes from a benign paraffin-embedded lymph node were used as an external diploid control. The DNA index was calculated as the ratio of the mode of the tumor $G_{0/1}$ peak to the mode of the diploid $G_{0/1}$ peak. A DNA index of 1.0 ± 0.1 was accepted as diploid. Cell cycle analysis was performed using area fit analysis.[18] The proliferative index was defined as the sum of $\%S + \%G_2$-M fractions.

RESULTS

Three of the four aspirates were very cellular; the fourth was moderately cellular (Table 2). All four included various admixtures of tissue fragments, loose cellular aggregates, and individual tumor cells (Fig 1). The fragments were irregularly shaped and consisted of densely packed tumor cells. Capillary-like blood vessels coursed through many of the fragments, especially the larger ones (Figs 1 and 2). This pattern was visible in both the Papanicolaou- and Diff-Quik–stained smears. As immediate alcohol fixation may allow cells to retain their natural configurations more readily than air-drying will, a greater contrast in cellular shapes between the neoplastic elements and the endothelial cells was apparent with the Papanicolaou stain; the latter were more elongated and slender than the tumor cells. Air-drying, on the other hand, accentuates differences in cellular sizes so a greater contrast in this regard between the two cell types was recognized in the Diff-Quik preparations. At times, jagged and cleft-shaped spaces were evident, and occasionally appeared to be lined by endothelial cells. Yet, staghorn-shaped blood vessels were not well developed.

Large areas of the smears were often occupied by monolayers of loosely cohesive and solitary tumor cells (Fig 3). Individual tumor cells were mononuclear with very high nuclear-cytoplasmic ratios (Fig 4). In two tumors, the neoplastic elements were quite uniform with oval to somewhat elongate nuclei with smooth borders. Moderate nuclear pleomorphism, including frequent notches and convolutions, characterized the other two, yet an ovoid configuration was pervasive (Fig 4). Pleomorphism in cellular size is more readily appreciated in the air-dried smears. Chromatin was finely granular to powdery and evenly distributed. One or two small but distinct nucleoli typically were evident. Mitotic figures were present in the aspirates of all four tumors. The cytoplasm was scanty, pale staining, and fragile. Vacuoles were obvious focally within the cytoplasm of the cells from two tumors. Another had cells with obvious focal cytoplasmic granularity. Cellular borders were variably evident.

All specimens also had small multicellular aggregates, often with a three-dimensional quality. Some resembled acinar or glandular structures, especially in the FNA from patient 1. Others were composed of spindle-shaped tumor cells with elongated nuclei and were organized in a concentric whorling or perithelial manner.

Histologically, all four tumors had large areas that were densely cellular and composed of relatively uniform-appearing mononucleated tumor cells. In two tumors, the neoplastic elements were predominantly ovoid, whereas a spindle configuration was more prominent in the other two. A perithelial array was focally present in all four tumors. In one, this created a striking resemblance to a *Zellballen* pattern. In three cases, staghorn-shaped vessels were well developed. Although most unusual, the sarcoma in patient 3 replaced thyroid parenchyma and appeared to be a primary thyroid sarcoma.

By electron microscopy, all four neoplasms were composed of densely

Table 1.—Clinical Features of Patients With Hemangiopericytoma

Patient No./Age, y/Sex/Race	Signs and Symptoms
1/83/M/W	Ascites, anemia, retroperitoneal mass
2/79/F/W	Ptosis, orbital mass
3/66/F/B	Rapidly enlarging thyroid mass
4/38/M/W	Rapidly enlarging neck mass, metastases on chest roentgenogram

Table 2.—Features of Hemangiopericytoma (HPC)*

Patient No.	Site of Primary HPC and FNA	Size of Primary Tumor, cm	Mitotic Figures per 10 HPF†	Original FNA Diagnosis
1	Retroperitoneum	12	4	Adenocarcinoma
2	Left orbit	2	13	Malignant small-cell nonlymphoreticular tumor (probably sarcoma)
3	Thyroid	8	6	Anaplastic carcinoma
4	Posterior neck area	?	11	Recurrent HPC

*FNA indicates fine-needle aspiration; HPF, high-power field.

†In the microscope that was used, the area of the HPF is 1.92 mm^2.

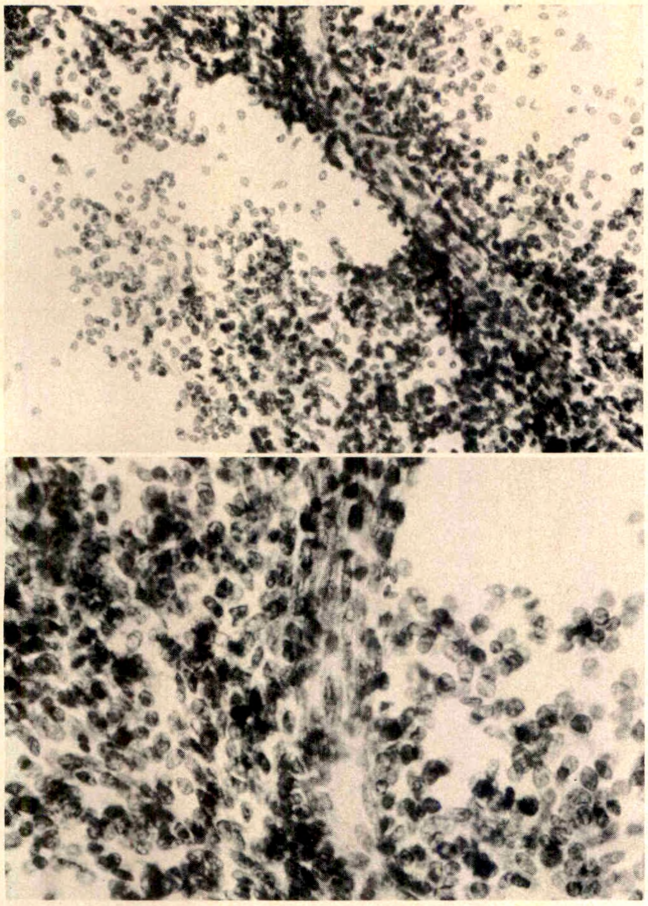

Fig 1.—Top, This cellular fine-needle aspirate includes aggregates of loosely cohesive and solitary tumor cells as well as large tissue fragments. The latter include a large vessel lined by endothelium distinct from the surrounding tumor cells (Papanicolaou, original magnification ×100). Bottom, Coursing through this cellular aggregate is a large vessel lined by elongated endothelial cells. The cells can be readily differentiated from the surrounding neoplastic pericytes (Papanicolaou, original magnification ×250).

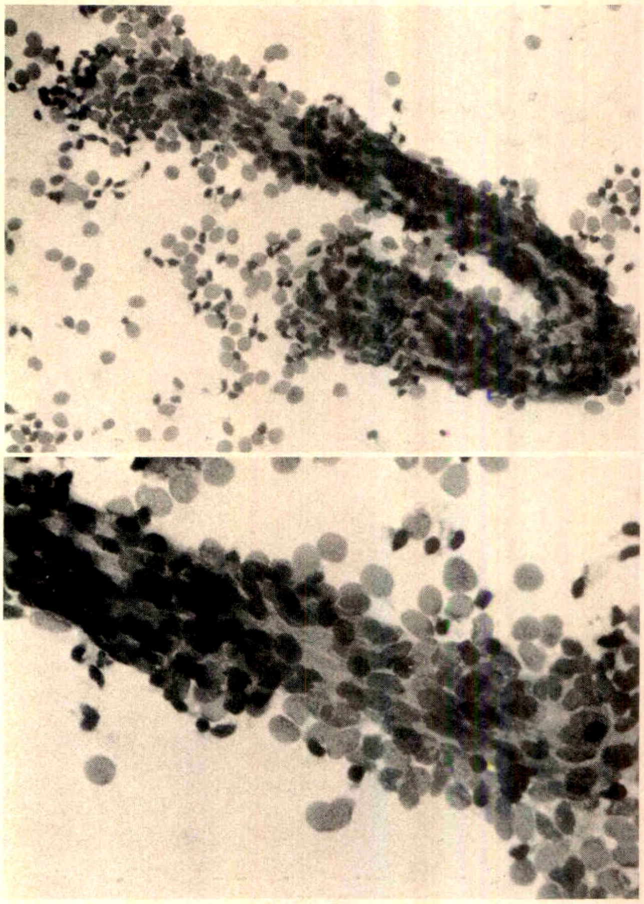

Fig 2.—Top, A large vessel is encased by uniform tumor cells. Similar-appearing neoplastic elements appear to be "falling off" the vessel (Diff-Quik, original magnification ×100). Bottom, Higher magnification shows two cell types. The larger tumor cells have oval nuclei and little in the way of visible cytoplasm. The smaller, more slender endothelial cells line the vessel; individual cells may also be present free of the vessel wall and admixed with the tumor cells (Diff-Quik, original magnification ×400).

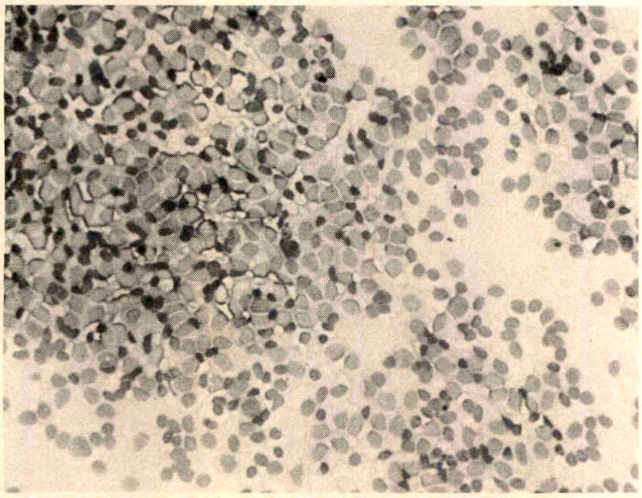

Fig 3.—A monolayer of very uniform mononucleated neoplastic cells with very high nuclear-cytoplasmic ratios appear cohesive but are not arrayed in any particular pattern (Diff-Quik, original magnification ×100).

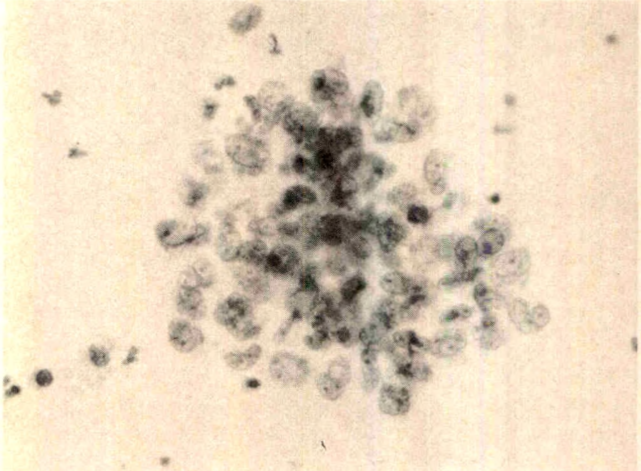

Fig 4.—This loosely cohesive aggregate consists of tumor cells with a single nucleus with small yet evident nucleoli. The irregular nuclear contours include notches and lobulations. The cytoplasm is indistinct (Papanicolaou, original magnification ×400).

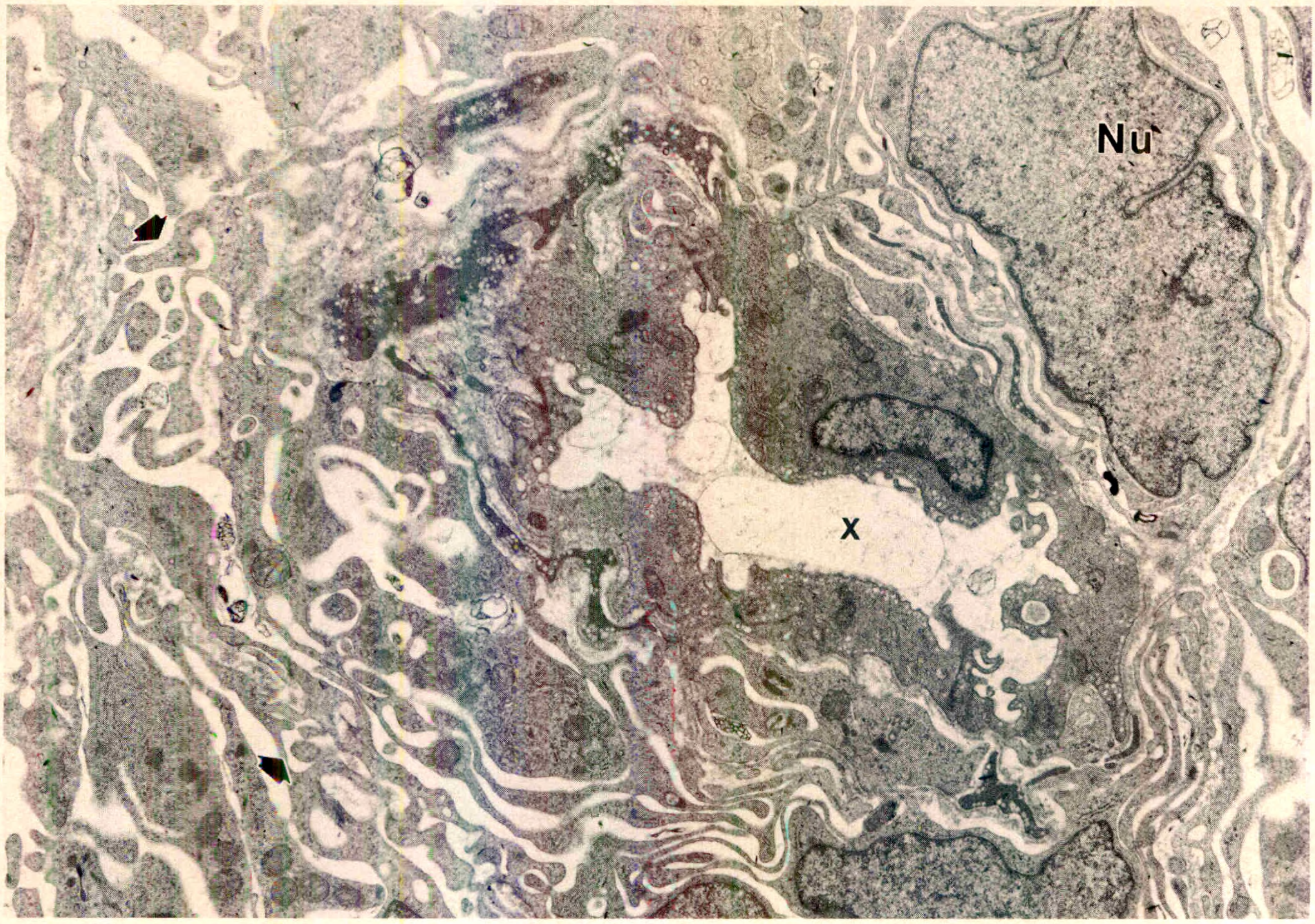

Fig 5.—Numerous delicate complex processes emanate from the neoplastic pericytes (arrows). Although many contacts are made, intercellular junctions are not evident. Focally, basal lamina–like material is scattered among the tumor cells. Note the distinct basal lamina (X) separating the capillary from the pericytes. Nu indicates a pericytic nucleus (uranyl acetate–lead citrate, original magnification ×9000).

packed mesenchymal elements. The orbital tumor from patient 2 was uniformly composed of relatively primitive mononucleated cells that were concentrically arrayed about capillarylike blood vessels (Fig 5). Their nuclei were encased by a thin shell of nondescript cytoplasms from which emanated two or more long, delicate, branching processes. Within these dendritelike processes, short bundles of filaments were seen. Numerous intercellular contacts were made among these processes, usually without any evidence of structural junctions. The basal lamina was diffuse but discontinuous. This corresponds to the type A pattern described by Battifora.[11] Ultrastructurally, the other three neoplasms were predominated by larger, plumper cells with less well-developed cellular processes; the latter were shorter, broader, and apparently less complex. Intercellular junctions were sparse and not well developed. In some cells, the cytoplasm contained greater volumes of organelles, especially rough endoplasmic reticulum. Cytoplasmic filaments were evident in all cases. The neoplasms from patients 1, 3, and 4 corresponded most closely to Battifora type B neoplasms. Type A development was definitely present in the neoplastic tissue from patients 1 and 3 and was strongly suggested in patient 4's neoplasm.

The tumor cells from patients 1 and 3 were aneuploid with DNA index values of 1.7 and 1.3, respectively. The tumor cells from patient 2 were diploid with a slight right-sided shoulder after the addition of benign lymphocytes; this could be due to a staining artifact. The proliferative indexes were 9.7%, 8.0%, and 8.9% for patients 1, 2, and 3, respectively. The mean coefficient of variation of the diploid peaks was 4.5, and the mean coefficient of variation of the tumor $G_{0/1}$ peaks was 7.1.

COMMENT

Hemangiopericytomas comprise a group of benign and malignant soft-tissue tumors that may arise in almost all body sites.[7-10] One fourth to one third of all HPCs arise in the lower extremities, especially the thigh. Approximately 10% to 25% are primary retroperitoneal tumors. An additional 10% to 16% arise in the head and neck region. Hemangiopericytomas occur predominantly in adults, with a relatively equal sex distribution. The majority of patients present with an enlarging mass lesion that may or may not be associated with pain.

Although not pathognomonic, the histologic hallmarks of HPCs include a relatively uniform population of mesenchymal tumor cells intimately arranged about a prominent vascular network, which includes irregularly shaped, dilated sinusoidal vessels. A major problem for the surgical pathologist in many instances is the inability to render an accurate assessment of the tumor's biologic potential.[7-10] No single histologic feature or constellation of microscopic findings provides universally agreed on prognostic criteria. Consequently, the authors of some of the larger published series have included a borderline category of HPCs.[7,9,10] Among the features that contribute to borderline or malignant diagnoses are the presence of moderate cellular pleomorphism, more than slight mitotic activity, tumor necrosis, and a high level of cellularity. The latter may lead to compression of some of the vascular lumina to the point that they are difficult to recognize. McMaster et al[7] stated that tumor cell configuration converted from spindled to ovoid and plump in the borderline and malignant categories. All four tumors in our series can be classified as malignant based on their high cellularity, brisk mitotic activity, and necrosis. In addition, moderate pleomorphism was present in two cases.

Specific ultrastructural features that identify tumor cells as showing definite pericytic differentiation do not occur. Yet, in a neoplasm with the light microscopic appearance of an HPC, the constellation cells with delicate, complex processes, prominent basal lamina about individual tumor cells, pinocytic vesicles, and cytoplasmic filaments are strongly supportive of such an interpretation.[11-13] These electron microscopic features were seen in all the cells examined from patient 2. These tumor cells were intimately arrayed about capillarylike vessels, but were separated from the latter by an intact basal lamina. Their long, thin cytoplasmic processes demonstrated complex interdigitation and intertwining. Yet, despite numerous intercellular contacts, structural junctions were absent or primitive at best. Although a few cells had prominent but small aggregates of cytoplasmic filaments, their differentiation closely resembled the type A tumors described by Battifora.[11] The other three tumors in our series included occasional cells with the above mentioned features and lacked structures indicative of other specific cell types, such as smooth-muscle elements. Neoplastic cells were plumper with less well-developed cellular processes that were shorter, broader, and less complex appearing. Greater amounts of organelles, especially rough endoplasmic reticulum, were present. These three cases correspond most closely to Battifora[11] type B tumors.

Whereas the popularity of aspiration cytology of many organs has accelerated recently in this country and elsewhere, experience with FNA of soft-tissue masses has been quite limited.[1-6] Most of the pertinent literature has originated in Scandinavia, and includes reports of large series with acceptable levels of diagnostic sensitivity and specificity. Rydholm et al[1] and Akerman et al[2] recently discussed two HPCs in their publications from the University Hospital of Lund (Sweden). One well-differentiated malignant HPC was interpreted as a benign mesenchymal tumor on FNA; perhaps a more radical surgical procedure would have followed a malignant cytologic diagnosis, but the patient showed no evidence of residual tumor after long-term follow-up. The aspirate from a histologically benign HPC from another patient was cytologically interpreted as a mesenchymal tumor, inconclusive as to its biologic potential. The FNA results were also recently described in the US pathology literature by Layfield et al.[3] Their report of 136 soft-tissue lesions included two histologically confirmed malignant HPCs. One of these was correctly diagnosed as such on the FNA; the other was cytologically interpreted as sarcoma, not otherwise specified. The cytologic features of the HPCs were not described in any of the above mentioned cases.

Two publications have specifically addressed the cytomorphological features of aspirated HPCs.[14,15] Nickels and Koivuniemi[14] reported their findings in five aspirated malignant HPCs, three of which were primary pulmonary neoplasms and two of which were from the retroperitoneum. Malignant cells were present in three of their FNAs; they were characterized by large, irregularly shaped, hyperchromatic nuclei with well-developed nucleoli, scanty cytoplasms, and mitotic figures. The cells from our two cases with moderate degrees of pleomorphism probably correspond to these cases; the nuclear variability in our cases was mostly of shape and not of size. Also, as in our material, tumor cells occurred in tissue fragments and as dispersed solitary elements. Intercellular cohesion was greatest near vascular spaces and vessels. Nickels and Koivuniemi[14] described the universal occurrence of endothelial-lined noblike projections of tumor cells. We were unable to identify such formations.

Nguyen and Neifer[15] also did not describe finding noblike projections. In their FNA report of two benign HPCs, uniform spindle-shaped tumor cells with benign chromatin patterns and inconspicuous nucleoli were present. In some respects, these features were present in our other two neoplasms; they consisted of very uniform, mononucleated cells with an evenly distributed chromatin pattern. Although the nucleoli were small, they were prominent, and the nuclei were more round to oval, rather than spindled and elongated, as described by Nguyen and Neifer.[15] Another important difference was the presence of mitotic figures in the aspirates from all our patients. The third case reported by Nguyen and Neifer[15] was a histologically malignant HPC; except for a larger size, the aspirated tumoral elements resembled those in the benign lesions, and a malignant tumor could not be diagnosed.

As pointed out by Koss et al,[6] an obvious major purpose of FNA of primary soft-tissue tumors is to distinguish between benign and malignant proliferations. In addition, a histogenetic or cellular typing of tumors is desirable. Based on our own experience and that which has been previously published, we believe that most malignant HPCs yield aspirates that are cellular, and are composed of cells with high nuclear-cytoplasmic ratios, mitotic figures, and mild to moderate pleomorphism. Often, this will permit an interpretation of a malignant neoplasm. However, as other authors have warned, this may not always be possible.[5,6,14,15] Even when an HPC is well sampled histologically, its biologic potential is not always readily evident.[7,9]

It may also be quite difficult to be certain that one is dealing with a mesenchymal tumor; in our own early experience, two of the primary neoplasms in our series were initially considered to be high-grade carcinomas in the FNA material. This especially may occur when an HPC arises in an unusual site such as the thyroid. However, the oval to elongate nuclear and cellular configurations point toward a mesenchymal differentiation. Other clues to such an interpretation include a diffuse, very finely granulated chromatin pattern and smooth, delicate nuclear membranes. In addition, intercellular cohesion but without evidence of epithelial differentiation is also suggestive of mesenchymal tumor. In one of our cases and in one previous report, focal acinuslike formations by the tumor cells have been seen, representing a potential diagnostic pitfall.[15] We were not able to make a specific diagnosis of HPC in any of our aspirates of primary neoplasms, although HPC was included in the differential diagnosis of patient 2. We believe that entertaining an interpretation of HPC in this situation is very difficult. Although Layfield et al[3] accurately diagnosed HPC in one patient, in most available reports the authors have not stated whether or not HPC was their initial cytologic diagnosis in the actual clinical setting. As reviewed by Enzinger and Weiss,[9] a number of other soft-tissue tumors may include areas that histologically mirror HPC in which tumor cells are intimately associated with large staghorn-shaped vessels. If the aspiration needle were to sample exclusively or predominantly such foci, then an incorrect interpretation might very well be rendered. On the other hand, in patients with histologically documented HPC, we believe that FNA may be very valuable and accurate in confirming recurrent or metastatic tumor.[6] As with other situations, review of previous surgical pathologic material at the time of FNA may prove extremely helpful in the decision-making process.

Analyses of soft-tissue tumors by flow cytometry have only infrequently been reported.[19-21] DNA aneuploidy has been shown to correlate with tumor grade and possibly with mortality. We are unaware of published studies of HPC by flow cytometry. Although no firm conclusions should be drawn from our small series, the results suggest that DNA aneuploidy may be related to prognosis in HPC. In the two cases in our series that were clearly aneuploid (patients 1 and 3), the patients died only 2 and 6 months, respectively, after diagnosis. The tumor with the highest DNA index (patient 1) was the tumor with the greatest pleomorphism and the greatest dissemination. The patient whose tumor was diploid was alive 54 months after the diagnosis. The proliferative indexes in all three cases were less than 10% and did not appear to be related to either ploidy or prognosis.

We wish to express our appreciation to Patt Moore for her expert assistance in the preparation of our manuscript.

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Detection of Epstein-Barr Virus Genomes in Archival Tissues by Polymerase Chain Reaction

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• We used oligonucleotide primers designed from DNA sequences unique to the long internal direct repeated region of Epstein-Barr virus (EBV) to enzymatically amplify this segment of the EBV genome in formalin-fixed, paraffin-embedded tissues. The products amplified from EBV templates were detected by hybridization with a labeled probe specific for this highly conserved, reiterated region. Epstein-Barr virus–related sequences were detected in the spleen of a patient with infectious mononucleosis, in lung and lymph node specimens from a patient with pulmonary manifestations of infectious mononucleosis, in various tissues from seven immunosuppressed organ transplant recipients with immunoproliferative disorders, and in small biopsy specimens from a patient with nasopharyngeal carcinoma. No viral sequences were detected in 20 histologically normal spleens or 10 lymph nodes. Polymerase chain reaction technology provides an effective means for documenting EBV infection in archival tissues. This approach should facilitate the diagnosis of posttransplant lymphoproliferative disorders and difficult cases of infectious mononucleosis and nasopharyngeal carcinoma.

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Epstein-Barr virus (EBV) is a member of the herpesvirus family that both actively and latently infects lymphoid cells. Epstein-Barr virus infection has been linked to various conditions, including infectious mononucleosis,[1,2] posttransplant lymphoproliferative disorders (PTLDs),[2,3] African Burkitt's lymphoma,[4] nasopharyngeal carcinoma (NPC),[5] and, most recently, Hodgkin's disease.[6-8] The repertoire of laboratory studies that can be used to detect EBV infection is limited, and diagnoses are often based on serologic evidence. Unique sequence probes from the Epstein-Barr virion are available to detect viral genomes by in situ hybridization and Southern blot analysis.[9-11] The logistical aspects that hinder the application of these technologies to diagnostic situations, however, include limited sensitivity and the requirement for fresh tissues. The enzymatic amplification of DNA sequences by polymerase chain reaction (PCR) technology provides a specific, rapid, and sensitive means for the detection of viral genomes. Furthermore, it can be readily applied to a variety of diagnostic materials, including archival, paraffin-embedded tissues.[12] We applied PCR technology to amplify a conserved region of the EBV genome to demonstrate viral sequences in archival tissues from diseases known to be associated with viral infection (ie, infectious mononucleosis, PTLDs, and NPC) and to determine whether normal lymphoid tissues contain detectable levels of latent virus.

MATERIALS AND METHODS

Cases were selected from the University Hospital's (Birmingham, Ala) surgical pathology and autopsy files to illustrate a spectrum of EBV-related conditions. All tissues had been routinely fixed in 10% formalin. Hematoxylin-eosin–stained slides were reviewed, and appropriate blocks were chosen for DNA extraction (see below). Clinical aspects such as age, diagnosis, and nature of therapeutic procedures were abstracted from surgical pathology reports and clinical records. DNA extracts from 20 histologically normal spleens (removed due to trauma or during staging laparotomies) and 10 histolog-

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ically normal lymph nodes were analyzed as normal controls.

DNA was extracted from single 8-μm sections of formalin-fixed, paraffin-embedded tissues as described by Shibata et al.[12] Briefly, sections were placed in a 1.5-mL microfuge tube, deparaffinized with xylene, rehydrated with alcohol, and then dried. Genomic DNA from the Raji cell line, derived from an EBV-infected African Burkitt's lymphoma, served as the positive control. Genomic DNA from a normal placenta was the negative control. Crude DNA extracts from an 8-μm section or 100 ng of control DNA were incubated in reaction mixtures that contained 10 mmol/L of TRIS hydrochloride buffer (pH 8.3), 50 mmol/L of potassium chloride, 1.5 mmol/L of magnesium chloride, 0.01% gelatin, 0.2 mmol/L each of deoxynucleotide triphosphates, 150 pmol of oligonucleotide primers, and 0.25 U of thermostable *Taq* polymerase (Cetus, Emeryville, Calif). The reactions were overlaid with mineral oil and subjected to 30 cyclesf amplification, each composed of 1 minute of denaturation at 94°C, 2 minutes at 46°C for annealing of primers and template, and 3 minutes at 72°C for synthetic extension of primers. Oligonucleotide amplimers designed from sequences in the long internal direct repeated region of EBV, prepared on an oligonucleotide synthesizer (Coder 300, Dupont, Wilmington, Del), were used to amplify a 110 base pair (bp) region[13] (Table 1). Nucleic acids present in the amplification reactions were precipitated, and the entire sample was subjected to electrophoresis on a 1.5% agarose gel (Seakem, FMC BioProducts, Rockland, Me). *Hind*III fragments of λ-DNA (New England Biolabs) and *Hae* III fragments of Phi X174 replicative form DNA (New England Biolabs) served as molecular weight standards. Nucleic acids were transferred onto hybridization membranes (GeneScreen Plus, Dupont) by alkaline blotting and detected with a [^{32}P]-labeled oligonucleotide probe complementary to EBV sequences flanked by the amplimers. Blots were prehybridized for 15 minutes in 1% bovine serum albumin, 1 mmol/L of ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 0.5 mmol/L of sodium phosphate (pH 7.2) and 7% sodium dodecyl sulfate (SDS) at 65°C and hybridized with the labeled probe in the same solution overnight at 65°C.[14] Blots were washed four times at 65°C in 1 mmol/L of EDTA, 40 mmol/L of sodium phosphate, 5% SDS, and 0.5% dried milk; four times at 70°C in 1 mmol/L of EDTA, 40 mmol/L of sodium phosphate, and 1% SDS; and twice at room temperature in 1 mmol/L of EDTA and 40 mmol/L of sodium phosphate. All washes were for 10 minutes in 200 mL of buffer. The blot was subjected to autoradiography on XAR-5 film (Eastman Kodak, Rochester, NY) at −85°C with an enhancing screen, typically for 2 to 4 hours. Tissues were scored as positive if the detecting probe annealed to a 110-bp fragment.

No cross-reactivity has been observed in DNA from cells infected with herpes simplex virus or cytomegalovirus in previous studies.[13] This assay has been shown to have a sensitivity to detect EBV sequences from three Raji cells, which contain approximately 50 genomes each.[13]

Extracts of paraffin sections from four normal lymph nodes were available as template for the control amplification of a cellular gene. A genomic segment in the 3′ flanking region of the apolipoprotein B gene was amplified as previously described.[15] Approximately 20% of material extracted from a paraffin section served as a source of target templates in the PCRs. Half of the reaction products were analyzed by Southern blotting and hybridization with a specific oligonucleotide probe.

RESULTS

Thirteen specimens from 10 patients were examined. They included samples of infectious mononucleosis syndromes (patients 1 and 2), PTLDs (patients 3 through 9), and NPC (patient 10). Pertinent clinical information and tissue sources are summarized in Table 2.

Patient 1 had serologically proved (positive slide test for infectious mononucleosis heterophile antibodies [Monospot, Ortho, Raritan, NJ] test) infectious mononucleosis and suffered splenic rupture. The spleen showed histologic features characteristic of infectious mononucleosis. The red pulp was expanded by a polymorphic lymphoid infiltrate that included prominent numbers of large activated lymphocytes and immunoblasts.

The clinical and pathologic features of patient 2 have been described in detail elsewhere.[16] This patient developed pulmonary complications during a heterophil antibody–positive syndrome. Titers of IgG antibodies to viral capsid antigen were 1:32 during his acute illness and 1:64 1 month later, when antibodies to Epstein-Barr nuclear antigen were 1:16. Histologic examination of lung and mediastinal lymph node specimens showed an infiltrate similar to that described in patient 1.

Patient 3 developed an infectious mononucleosis syndrome after receiving a renal allograft. Subsequently, his heterophil antibody titer was elevated. Along with a deteriorating function of

Table 1.—Oligonucleotides for Polymerase Chain Reaction of Epstein-Barr Virus (From *Bam*Hi W Region[9])

Oligonucleotide No.	Sequence
Primers	
1041-1060	5′GTTCGCGTTGCTAGGCCACC3′
1131-1150	5′AGGACCACTTTATACCAGGG3′
Probe	
1071-1110	5′AGCGCTTTTACGTAAGCCAGACAGCAGCCAATTGTCAGTT3′

Table 2.—Summary of Patients With Documented Epstein-Barr Virus (EBV) Infection*

Patient No./ Age, y	Underlying Condition	Source of Specimen
1/17	Infectious mononucleosis	Spleen
2/48	Infectious mononucleosis	Lung, mediastinal node
3/17	Renal transplant; PTLD†	Kidney, lymph node, skin biopsy
4/29	Renal transplant; PTLD	Lymph node
5/15	Heart-lung transplant; PTLD	Colonic biopsy
6/35	Insulin-dependent diabetes; renal and pancreas transplant; PTLD	Lung (autopsy)
7/36	Heart transplant: PTLD	Lung biopsy
8/58	Heart transplant; PTLD	Spleen (autopsy)
9/44	Renal transplant: PTLD	Kidney (autopsy)
10/19	Nasopharyngeal carcinoma	Biopsy of nasopharyngeal tumor

*Results of polymerase chain reaction assays were abnormal for all patients.
†PTLD indicates posttransplant lymphoproliferative disorder.

the renal allograft, disseminated lymphadenopathy, cytopenias, and a rash were noted. Furthermore, an abdominal lymph node, as well as the transplanted kidney and skin, had an intense infiltrate of immunoblastic cells that had a T-lymphocyte immunophenotype and lacked expression of immunoglobulin light chains. Biopsy specimens from patients 4 and 5 were similar and showed sheets of atypical immunoblasts that were indistinguishable from those of immunoblastic lymphoma by morphologic criteria. Immunophenotypic analysis of tissue from patient 3 showed polyclonal expression of κ and λ light chains, while that from patient 4 showed a light chain–restricted monoclonal proliferation. The main histologic finding in all specimens from patients 6 through 9 was a diffuse polymorphic lymphoid infiltrate with variable numbers of atypical large cells.

Patient 10 had a typical undifferentiated NPC (ie, lymphoepithelioma) composed of scattered clusters of malignant epithelial cells surrounded by an infiltrate of small lymphocytes.

Amplification of Raji cell DNA showed the predicted 110-bp product on ethidium bromide–stained agarose gels. Southern blot analysis confirmed that the amplification products from all cases were derived from an EBV genomic template based on the presence of a 110-bp species that annealed to the detecting oligonucleotide probe under high-stringency conditions. The results of representative positive and negative specimens are shown in Figs 1 and 2. An identical band was detected in the amplification products from the tissue sections and genomic DNA from the Raji lymphoblastoid cell line. No EBV-related sequences were present in amplification reactions from the 30 control lymphoid tissues (10 normal spleen and 20 normal lymph node specimens).

Extracts from 4 lymph nodes were tested for the ability to serve as a template for the amplification of a cellular genomic target. A segment in the 3′ flanking region of the apolipoprotein B gene was successfully amplified in three of four of the paraffin extracts.

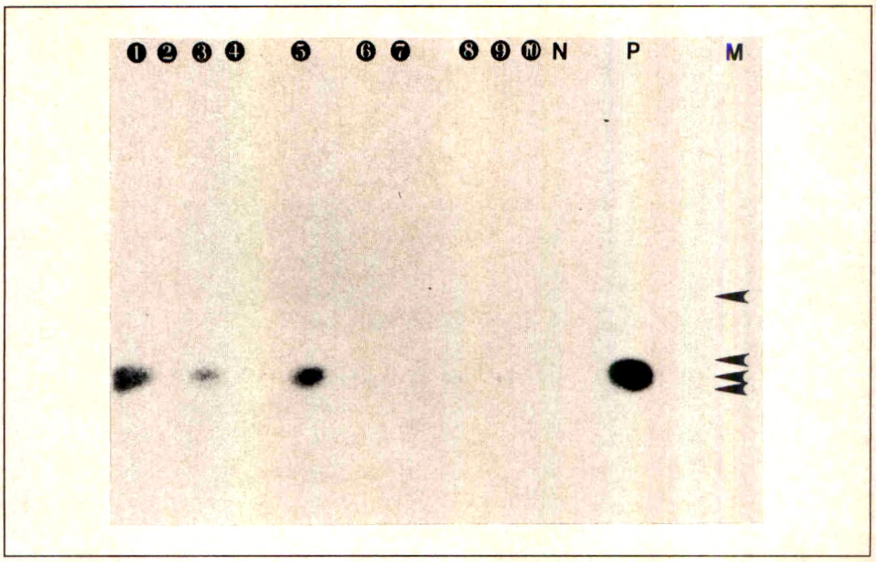

Fig 1.—Southern blot analysis of representative positive and negative polymerase chain reaction amplification reactions. Lane 1 represents patient 4 (posttransplant lymphoproliferative disorder); lane 3, patient 10 (nasopharyngeal carcinoma); lane 5, patient 3 (posttransplant lymphoproliferative disorder); lanes 2, 4, and 6 through 10, normal tissues; lane N, normal placental DNA; and lane P, Raji cell DNA. The migration of *Hae* III fragments of Phi X174 replicative form detected by homidium bromide staining is shown in lane M (from top, 234 base pairs [bp], 194 bp, 118 bp, and 71 bp).

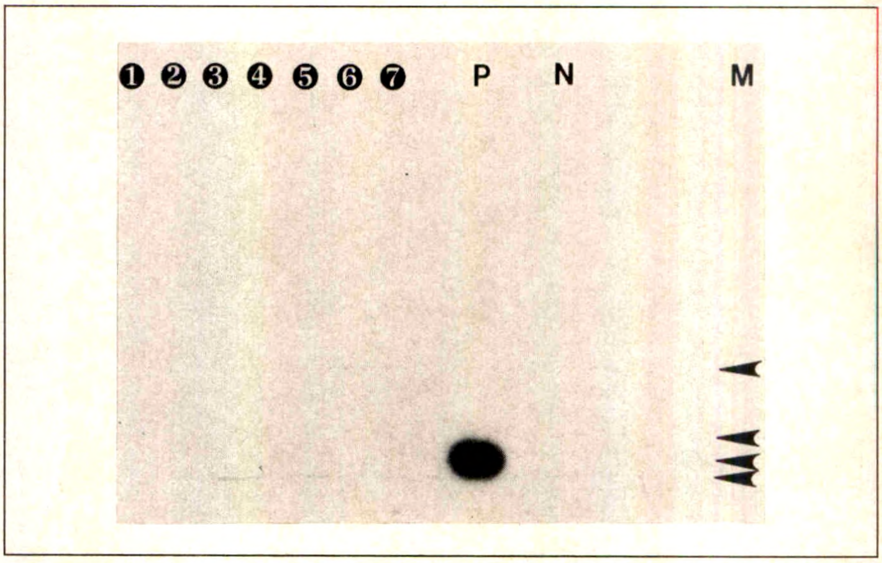

Fig 2.—Southern blot analysis of polymerase chain reaction amplification products from normal lymphoid tissues. Amplifications and blotting were performed as described in Fig 1. Lanes 1 through 7 show results from the spleen; lane P, Raji cell DNA; and lane N, normal placental DNA. The migration of *Hae* III fragments of Phi X174 replicative form is shown in lane M.

COMMENT

Polymerase chain reaction technology provides a sensitive, specific, rapid method for detecting EBV infection in routinely processed tissues. Viral sequences from as few as three Raji cells (approximately 50 genomes per cell) are detectable by this approach.[13] Therefore, it is estimated that the presence of several EBV-infected cells in single paraffin sections should be within the limits of detection by PCR technology. In situ hybridization and Southern and slot blotting analysis with labeled viral probes have also been used to detect EBV in human tissues. Polymerase chain reaction technology is an alternative that does not require the extraction of genomic DNA from fresh tissues and can be applied to small biopsy specimens that have been routinely processed. The interpretation of PCR assays is objective and independent of morphologic analysis.

We detected EBV genomes in the spleen from a patient with infectious mononucleosis by PCR. With the use of in situ hybridization, Weiss and Movahed[2] failed to detect viral sequences in spleens from two patients with infectious mononucleosis. They postulated that this could be caused by technical considerations, such as nucleic acid degradation or unavailability of viral genomes for hybridization, by a lack of sensitivity, or by the true absence of EBV-infected cells.[2] Based on the PCR data, it is likely that EBV-infected cells reside in tissues that demonstrate the characteristic lymphoid reaction but are infrequent or have a content of viral genomes below the level of discrimination for in situ hybridization.

The detection of EBV genomes by PCR may be helpful in several diagnostic situations. Posttransplant lymphoproliferative disorders have emerged as potentially life-threatening complications in transplant recipients taking immunosuppressive agents. These disorders include a spectrum of lymphoproliferative lesions that overlap clinically and pathologically with malignant lymphomas.[17] Recognition of these disorders is important because many patients will recover with modulation of their immunosuppressive therapy.[17] Our study suggests that PCR can demonstrate EBV genomes, even in small biopsy specimens, thus corroborating the diagnosis of PTLD.

Detection of EBV sequences could also be useful in distinguishing infectious mononucleosis from malignant lymphoma. Infectious mononucleosis results from acute EBV infection and is generally a self-limited illness in immunocompetent individuals. Typically, the diagnosis is based on clinical features and a positive Monospot test, but biopsies are occasionally performed in some patients. The characteristic pathologic abnormality is lymphoid hyperplasia that primarily affects lymph nodes but may also involve extranodal sites. This hyperplasia is characterized by a polymorphic proliferation of large transformed lymphocytes, small lymphocytes, and plasma cells, and may exhibit extreme atypia. In some instances, monomorphic expansions of large transformed cells arranged in sheets and clusters may simulate malignant lymphoma.[18] Demonstration of EBV genomes would suggest a diagnosis of infectious mononucleosis in this context, as it did in two of our patients.

Clonal EBV genomes have been detected in NPC,[11] and patients frequently have elevated EBV antibody titers at the time of diagnosis.[19,20] Demonstration of EBV infection in biopsy specimens may provide additional evidence of an undifferentiated NPC, particularly in patients who present with metastases to cervical lymph nodes from an occult primary tumor.

In summary, PCR is a rapid, sensitive, and specific method for detecting EBV genomes in archival tissues. The ability to detect viral genomes in each of the diseases known to be associated with EBV infection demonstrates the utility of this approach. Epstein-Barr virus–related sequences were not detected in DNA from sections of normal spleen and lymph node, suggesting that latent infection of lymphoid cells is below the limits of discrimination of this technology.

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Heterogeneity of Common Hematologic Parameters Among Racial, Ethnic, and Gender Subgroups

Sunita Saxena, MD, Edward T. Wong, MD

• This study was performed to determine whether hematologic indexes might show heterogeneity among different subgroups of the population and, if differences were found, to develop reference ranges for each subgroup. Complete blood cell counts and leukocyte differential cell counts were compared in healthy white (n = 663), black (n = 697), Latin-American (n = 535), and Asian (n = 247) adults. Women had significantly lower red blood cell counts, hemoglobin levels, and hematocrits than men. In addition, women in at least three racial/ethnic groups, had higher platelet, white blood cell, and granulocyte counts and lower monocyte counts than men. Among blacks and Latin Americans, women had lower mean corpuscular volumes, mean corpuscular hemoglobin levels, and mean corpuscular hemoglobin concentrations than men. The following racial/ethnic differences were found: higher red blood cell distribution widths and lymphocyte and monocyte counts, and lower hemoglobin levels, hematocrits, mean corpuscular hemoglobin levels, mean corpuscular hemoglobin concentrations, and white blood cell, granulocyte, and band counts among blacks; higher white blood cell, granulocyte, and band counts among Latin Americans; and higher red blood cell and lower lymphocyte and monocyte counts among Asians than one or more of the other three groups. We concluded that the complete blood cell counts and leukocyte differential cell counts exhibit marked heterogeneity and that reference ranges that reflect the proper racial, ethnic, and sex group should be developed for these parameters.

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Physicians rely on reference ranges to interpret laboratory data on their patients. However, providing reference ranges appropriate for an individual patient is a far more complex task than is generally appreciated by physicians who use laboratory data or by laboratorians who provide the test results and accompanying reference ranges. While most clinical laboratories use hematology reference ranges that reflect well-recognized gender differences for red blood cell (RBC) counts, hemoglobin (Hb) levels, and hematocrits (Hct), only single reference ranges are used for other hematologic parameters, such as white blood cell (WBC) counts, mean corpuscular volumes (MCVs), and platelet counts, etc. Even reference sources, such as Wintrobe's *Clinical Hematology*,[1] provide only one reference range for hematologic indexes other than RBC counts, Hb levels, and Hct values. In addition, many of those reference ranges were based on data that are ancient and laboratory procedures that are now outdated.[2,3] Furthermore, there is marked scarcity of comparative studies among different racial, ethnic, and gender subgroups. Most of the previous studies of peripheral blood cell counts have been restricted to a single subgroup of the population, such as women,[4-6] children,[7,8] or a single racial or ethnic subgroup.[9,10]

Since this hospital serves a mixture of different racial and ethnic groups, this study was performed to determine whether hematologic indexes might show heterogeneity among different subgroups of the population similar to what had been previously demonstrated for clinical chemistry data[11-13] and, if differences were found, to develop appropriate reference ranges for each subgroup. We compared commonly used hematologic parameters in the men and women from white, black, Asian, and Latin-American backgrounds.

SUBJECTS AND METHODS

During the period of December 1985 through July 1988, employees of this medical center who were attending the Employee Health Service for their annual health-screening survey or preemployment physical examination were recruited with their informed consent as study subjects. They underwent screening by the nursing staff on the basis of their responses to a health history questionnaire and the results of their physical examination to exclude those with significant illness. These subjects constituted our presumably healthy population. Regular users of medications, including, but not limited to, aspirin, birth control pills, anticonvulsants, and vitamin supplements, were excluded from the analyses. Based on self-identification, employees from white, black, Asian, and Latin-American backgrounds were included in the study. The last named group included subjects whose origins were from Mexico, South/Central America, Cuba, Puerto Rico, or other Spanish cultures, regardless of their race. The Orientals and Pacific Islanders were included in the Asian group.

Laboratory Procedures

All subjects were rested before a phlebotomy. Blood was collected from each subject into a lavender-top tube that contained liquid sodium edetic acid (EDTA) and sent for a complete blood cell (CBC) count and a manual leukocyte differential cell count. In the initial part of the study, a manual differential

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cell count that included lymphocytes, monocytes, eosinophils, basophils, band cells, and segmented neutrophils was performed on all samples. One hundred cells were counted in the manual differential cell count. Unfortunately, in the middle of the study, the hematology laboratory upgraded its equipment so that it could provide automated three-part differential cell counts as part of CBC counts. In the three-part differential cell count, eosinophils, basophils, band cells, and segmented neutrophils were all combined under one category of granulocytes that were reported, along with lymphocytes and monocytes. During the transition period, both the manual and the automated differential cell counts were performed; however, subsequently manual differential cell counts were no longer done on a routine basis. As a result of this change, eosinophil, basophil, band cell, and segmented neutrophil counts were available only during the initial part of the study, and were replaced later on by granulocyte counts. All differential cell counts (in percentages) were converted into absolute cell counts.

The CBC counts, including the RBC and WBC counts, Hb level, Hct value, MCV, mean corpuscular hemoglobin level, mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width, platelet count, and mean platelet volume (MPV), and the three-part differential cell count were performed on a Coulter hematology analyzer (Model S Plus IV, Coulter Diagnostics, Hialeah, Fla) according to the manufacturer's instructions. All Coulter-generated platelet counts were confirmed by visual platelet estimates of the blood smear. The CBC counts were performed between 30 minutes and 1 hour following a phlebotomy.

The Coulter counter was calibrated twice each year, following the preventive maintenance inspection. In addition, recalibration was done whenever a component that involved the dilution characteristics or the primary measurements was replaced. The calibration procedure used a kit (S-Cal Calibrator Kit supplied by Coulter Diagnostics). Each day, quality control, as suggested by the manufacturer, was performed.

Statistical Analyses

The results in the eight subgroups (males and females from four racial and ethnic groups) were compared by using the Mann-Whitney *U* test. The reference ranges were developed nonparametrically by using the 2.5th and 97.5th percentiles of the distribution. These nonparametric methods of comparison required no assumptions about the distribution of frequencies that were often nongaussian. A two-tailed *P* value of <.05 was considered to be significant. Multiple comparisons were done, as Dunn[14] has recommended, to minimize recognition of spurious results.

RESULTS

During the study period, 2142 employees of this medical center who ranged from 16 to 67 years of age were included. Thirty-one percent were white, 33% were black, 25% were Latin American, and 11% were Asian.

The results are presented in the form of means and SDs in Tables 1 and 2 only to provide the reader with some information regarding the distribution and spread of the data. Tables 1 and 2 should not be used to develop reference ranges because of the nongaussian distribution

Table 1.—Comparison of Complete Blood Cell and Differential Cell Counts in Men*

	Mean ± SD			
	White	Black	Latin American	Asian
Test				
n	181	172	141	72
WBCs, 10^9/L	6.1 ± 1.8†	6.0 ± 1.8‡	6.9 ± 1.9§	6.3 ± 2.0
RBCs, 10^{12}/L	5.0 ± 0.3‖	4.9 ± 0.5‡	5.0 ± 0.3	5.2 ± 0.4¶#
Hb, g/L	153 ± 9†‖	147 ± 11‡	155 ± 9	154 ± 10¶
MCHC, g/L	341 ± 7‖	338 ± 7‡	341 ± 7	339 ± 7#
Plt, 10^9/L	270 ± 5†	272 ± 60‡	285 ± 55§	305 ± 56¶#
MPV, fL	9.3 ± 0.9	9.5 ± 1.1	9.4 ± 0.9§	9.0 ± 0.9¶#
Hct	0.44 ± 0.02†‖	0.43 ± 0.03‡	0.45 ± 0.02	0.45 ± 0.02¶#
MCV, fL	89.3 ± 3.9	88.4 ± 6.3	89.6 ± 4.2	88.6 ± 6.5
MCH, pg	30.5 ± 1.5‖	29.9 ± 2.4‡	30.5 ± 1.6	30.0 ± 2.5
RDW, %	13.0 ± 0.8†‖	13.6 ± 1.1‡	13.2 ± 0.6§	13.0 ± 0.7¶
3-part differential cell count				
n	106	114	101	46
Lymphocytes, 10^6/L	2011 ± 502	2053 ± 532	2150 ± 603	1993 ± 633
Monocytes, 10^6/L	366 ± 140	369 ± 140	385 ± 137	359 ± 199
Granulocytes, 10^6/L	3645 ± 1585†	3569 ± 1524‡	4389 ± 1731	4215 ± 1815¶#
Manual differential cell count				
n	80	72	48	32
Segmented neutrophils 10^6/L	3841 ± 1462†	3660 ± 1654‡	4431 ± 1400	3782 ± 981
Band cells, 10^6/L	16 ± 30‖	5 ± 19‡	33 ± 60	20 ± 88
Lymphocytes, 10^6/L	1927 ± 543	1995 ± 620	2091 ± 929	2060 ± 936
Monocytes, 10^6/L	231 ± 158	284 ± 207	255 ± 195	216 ± 188
Eosinophils, 10^6/L	130 ± 130	138 ± 128	173 ± 244	148 ± 173
Basophils, 10^6/L	34 ± 52	27 ± 41	34 ± 54	52 ± 49¶

*WBCs indicate white blood cells; RBCs, red blood cells; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Plt, platelets; MPV, mean platelet volume; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; and RDW, red blood cell distribution width.

†White vs Latin American, *P* < .05.

‡Black vs Latin American, *P* < .05.

§Latin American vs Asian, *P* < .05.

‖White vs black, *P* < .05.

¶Black vs Asian, *P* < .05.

#White vs Asian, *P* < .05.

of results for a number of tests. The results of racial and ethnic comparison are also included in Tables 1 and 2.

Within each racial and ethnic group, men were also compared with women (not shown). Regardless of the racial or ethnic background, women had significantly lower RBC counts, Hb levels, and Hct values than men. Black women and Latin-American women also had significantly lower MCVs, MCHCs, and mean corpuscular hemoglobin levels than their male counterparts. No such difference was noted in other racial/ethnic groups. Among Latin Americans, blacks, and Asians, women had significantly lower monocyte counts, and among whites, blacks, and Latin Americans, women had higher platelet and WBC counts than men.

The reference ranges in men and women are shown in Tables 3 and 4.

COMMENT

Analyses of 2142 CBC counts and leukocyte differential cell count results showed that there was a marked heterogeneity among racial, ethnic, and gender subgroups of an apparently healthy population.

Sex Differences

With the exception of Asian women, women had higher mean WBC and platelet counts than men. Higher granulocyte counts among women were largely responsible for the difference in the WBC counts. One earlier report found a significant sex difference in total leukocyte and neutrophil counts[15]; however, that study was done on subjects of European origin only. Blacks, as well as Latin Americans, also exhibited a similar sex difference in WBC and granulocyte counts. No sex difference was noted in the manual differential cell count results. However, there was a sex difference in Coulter-generated monocyte counts. The women from black, Asian, and Latin-American backgrounds had significantly lower automated monocyte counts than men. Since this sex difference in monocyte counts was not confirmed by the manual counts and the Coulter counter only approximated the number of monocytes, the meaning of this finding was questionable.

The results of this study also confirm the findings of earlier reports on differences in platelet counts. In one study of black, white, and Latin-American subgroups, women were found to have higher platelet counts than men.[16] A higher prevalence of iron deficiency among women was the most likely explanation for the observed sex difference in the platelet counts in this study. In women from all groups, transferrin saturation was significantly lower when compared with that in men. The details of the transferrin saturation study will be published separately at a later date. Bailey and associates[17] showed that iron status, as determined by transferrin saturations, was less than adequate (<.16) in 2% of the males compared with 12% of the females in a study done on 193

Table 2.—Comparison of Complete Blood Cell and Differential Cell Counts in Women*

	Mean ± SD			
	White	Black	Latin American	Asian
Test				
n	482	525	394	175
WBCs, 10^9/L	6.6 ± 1.8†	6.5 ± 2.0‡	7.1 ± 1.9§	6.2 ± 1.5‖
RBCs, 10^{12}/L	4.4 ± 0.3	4.4 ± 0.4	4.4 ± 0.3	4.4 ± 0.4
Hb, g/L	134 ± 10†¶	128 ± 10‡	132 ± 11	133 ± 10#
MCHC, g/L	341 ± 6†¶	335 ± 8‡	340 ± 31	339 ± 8‖#
Plt, 10^9/L	300 ± 62†¶	319 ± 73	315 ± 69	302 ± 60#
MPV, fL	9.4 ± 0.9	9.5 ± 1.0	9.4 ± 0.9§	9.0 ± 0.8‖#
Hct	0.39 ± 0.02¶	0.38 ± 0.03‡	0.39 ± 0.03	0.39 ± 0.02#
MCV, fL	89.7 ± 4.9†¶	87.1 ± 5.9‡	88.2 ± 5.8§	89.5 ± 5.9#
MCH, pg	30.6 ± 1.9†¶	29.9 ± 2.3‡	29.9 ± 2.3§	30.3 ± 2.4#
RDW, %	13.0 ± 0.8†¶	13.8 ± 1.2	13.3 ± 1.1§	13.0 ± 1.2#
3-part differential cell count				
n	284	375	295	130
Lymphocytes, 10^6/L	1972 ± 541†¶	2146 ± 553	2112 ± 616§	1865 ± 466#
Monocytes, 10^6/L	366 ± 155	336 ± 150	322 ± 220	281 ± 109‖#
Granulocytes, 10^6/L	4125 ± 1545†¶	3834 ± 1626‡	4570 ± 1662§	3807 ± 1179
Manual differential cell count				
n	216	175	114	50
Segmented neutrophils, 10^6/L	4402 ± 1495¶	4037 ± 1618‡	4692 ± 1467	4296 ± 1329
Band cells, 10^6/L	16 ± 37¶	7 ± 24‡	22 ± 58	9 ± 32
Lymphocytes, 10^6/L	2005 ± 584†¶	2157 ± 664	2168 ± 650	1993 ± 409
Monocytes, 10^6/L	300 ± 234	277 ± 173	288 ± 170	238 ± 158
Eosinophils 10^6/L	123 ± 156	128 ± 227	153 ± 322	128 ± 144
Basophils, 10^6/L	37 ± 49	33 ± 62	33 ± 52	33 ± 47

*For explanation of abbreviations, see footnote 1 to Table 1.
†White vs Latin American, $P < .05$.
‡Black vs Latin American, $P < .05$.
§Latin American vs Asian, $P < .05$.
‖White vs Asian, $P < .05$.
¶White vs black, $P < .05$.
#Black vs Asian, $P < .05$.

Table 3.—Hematologic Reference Ranges (2.5th to 97.5th Percentiles) in Men*

	Range			
	White	Black	Latin American	Asian
Test				
n	181	172	141	72
WBCs, 10^9/L	3.7-10.4	3.5-9.6	4.1-11.5	3.4-11.5
RBCs, 10^{12}/L	4.3-5.8	4.0-5.8	4.4-5.6	4.0-6.2
Hb, g/L	130-173	119-167	137-170	125-170
MCHC, g/L	324-354	321-352	325-356	321-353
Plt, 10^9/L	176-372	167-408	176-397	223-422
MPV, fL	7.7-11.2	7.5-12.4	7.4-11.3	7.4-10.9
Hct	0.38-0.49	0.36-0.48	0.40-0.50	0.36-0.50
MCV, fL	81.6-96.6	71.8-99.8	82.0-96.5	67.3-96.3
MCH, pg	27.2-33.4	23.3-33.9	26.7-33.2	21.6-33.4
RDW, %	11.9-14.3	12.1-16.2	12.1-14.7	11.8-14.6
3-part differential cell count	106	114	101	46
n				
Lymphocytes, 10^6/L	1260-3046	1203-3166	1117-3358	1030-3381
Monocytes, 10^6/L	133-662	144-774	135-682	83-767
Granulocytes, 10^6/L	1781-7715	1492-6561	2412-8326	1509-7257
Manual differential cell count				
n	80	72	48	32
Segmented netrophils	1505-7004	1110-6697	2400-7592	2016-5503
Band cells, 10^6/L	0-68	0-72	0-208	0-63
Lymphocytes, 10^6/L	650-2800	969-3303	940-4224	897-3500
Monocytes, 10^6/L	0-512	21-831	0-684	0-528
Eosinophils, 10^6/L	0-424	0-467	0-804	0-315
Basophils, 10^6/L	0-156	0-162	0-168	0-140

*For explanation of abbreviations, see footnote 1 to Table 1.

Table 4.—Hematologic Reference Ranges (2.5th to 97th percentiles) in Women*

	Range			
	White	Black	Latin American	Asian
Test				
n	482	525	394	175
WBCs, 10^9/L	3.8-10.6	3.4-11.2	4.1-11.8	3.5-9.7
RBCs, 10^{12}/L	3.8-5.0	3.6-5.3	3.7-5.1	3.7-5.4
Hb, g/L	114-155	106-149	103-151	113-150
MCHC, g/L	327-353	319-350	320-353	322-355
Plt, 10^9/L	188-438	193-485	198-460	193-417
MPV, fL	7.7-11.5	7.7-11.6	7.8-11.5	7.6-11.1
Hct	0.34-0.45	0.31-0.44	0.31-0.44	0.33-0.44
MCV, fL	78.0-98.0	72.9-97.5	69.6-95.8	72.4-97.0
MCH, pg	25.9-33.8	23.7-33.1	22.3-33.0	22.1-33.8
RDW, %	11.7-15.2	12.0-17.3	11.9-16.4	11.6-16.6
3-part differential cell count				
n	284	375	295	130
Lymphocytes 10^6/L	1139-3185	1283-3292	1066-3444	936-2746
Monocytes, 10^6/L	100-743	104-666	108-670	87-510
Granulocytes, 10^6/L	2025-7457	1434-7678	2193-8241	1931-6131
Manual differential cell count				
n	216	175	114	50
Segmented neutrophils, 10^6/L	2025-7332	1502-8142	1854-7574	1600-7326
Band cells, 10^6/L	0-130	0-86	0-132	0-107
Lymphocytes, 10^6/L	1008-3381	1045-3477	890-3731	1235-2592
Monocytes, 10^6/L	0-819	21-717	63-656	0-651
Eosinophils, 10^6/L	0-518	0-456	0-497	0-492
Basophils, 10^6/L	0-161	0-196	0-150	0-156

*For explanation of abbreviations, see footnote 1 to Table 1.

black and Spanish-American subjects.

Although there was a sex difference in the platelet counts, we did not see a similar phenomenon for the MPVs. This was in contrast to that found in an earlier study that demonstrated an inverse relationship between platelet counts and MPV.[18] In that study, platelet counts were found to be higher and MPVs to be lower in females.

Gender differences with regard to RBC counts, Hb levels, and Hct values are well documented. However, we found differences between men and women for MCVs, mean corpuscular hemoglobin levels, and MCHCs as well. These parameters were significantly lower in black women and Latin-American women.

Racial and Ethnic Differences

Blacks.—Black men and women had much higher red blood cell distribution widths than did men and women from the other three groups. Hemoglobin levels, Hct values, mean corpuscular hemoglobin levels, and MCHCs were also lower in blacks (both men and women) when compared with whites and Latin Americans. Similar differences of Hb levels, Hct values, and MCHCs between blacks and Spanish Americans have been reported earlier.[17] Because these racial and ethnic differences were seen in both sexes, genetic factors, such as hemoglobin S and α-thalassemia, seemed to be likely although nutritional factors might also play a role.

In comparison with whites and Latin Americans, black men and women also had lower WBC, granulocyte, segmented neutrophil, and band cell counts. The results of this study support earlier reports of lower leukocyte counts in blacks than in whites.[19]

Among women, the platelet counts were significantly higher in blacks than in whites. This could also be attributed to a higher prevalence of iron deficiency among black women since they had a higher red blood cell distribution width and lower MCV in comparison with white women. Among men, too, the mean platelet count in blacks was higher than in whites, but the difference was not statistically significant. This is in contrast to the findings of a study done in London, England, that demonstrated higher platelet counts in white than in black men. No explanation for that finding was offered, however.[20]

Latin Americans.—In this study, WBC counts in Latin Americans were significantly higher than in the other three groups. Higher segmented neutrophil, band cell, and granulocyte counts were responsible for the differences in the WBC counts. We were not aware of reports of higher leukocyte counts in Latin Americans than in whites, blacks, and Asians.

Asians.—While Asian men had significantly higher RBC and granulocyte counts than white men and black men, Asian women had the lowest mean WBC and granulocyte counts. The MPV results were significantly lower among Asian men and women. However, findings regarding platelet counts were inconsistent. While Asian men had the highest platelet counts when compared with other men, Asian women had platelet counts that were lower than at least two other groups (black and Latin-American women). The reasons for this discrepancy were not clear. The mean lymphocyte (Coulter generated) and monocyte counts among Asian men, as well as women, were lower than all the other groups compared although these differences were significant only between black women and Asian women.

Reference Ranges

Because of the heterogeneity of common hematologic indexes in different racial, ethnic, and gender groups, it would be inappropriate to combine all subgroups and derive a single reference range for each parameter. Furthermore, reference ranges provided by the manufacturer of an instrument or in the literature are not suitable, especially in a multiracial population since many studies on reference ranges were done by using an exclusively white population and outdated methods. Consequently, we have developed reference ranges for CBC counts and leukocyte differential cell counts that reflect proper racial, ethnic, and sex group and current methodology.

We conclude that the routinely measured hematologic indexes exhibit racial, ethnic, and gender differences. Reference ranges should reflect these differences.

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Teaching Pathology and Laboratory Medicine

And gladly would he learn and gladly teach.
CHAUCER, *Canterbury Tales*

On the Fifth Year, Subspecialty Certification, and Delayed Gratification

A Partial Solution

John C. Neff, MD

In 1985 the American Board of Pathology (ABP) added, as a prerequisite to sit for its basic qualifying examinations, a "certifying" year, thus increasing the basic pathology residency by 1 year if the applicant enters residency directly on graduation from medical school.[1] The Accreditation Council on Graduate Medical Education via the Residency Review Committee for Pathology continues to accredit basic pathology programs in combined anatomical and clinical pathology (AP/CP) for only 4 years. The extra certifying year has become known as the "fifth year," although of course it might be the fourth year if the applicant were to pursue a straight AP or CP residency.

It would surely be an understatement to say that this decision by the ABP has caused a great deal of confusion, hostility, and resentment among pathology chairmen, residents, and program directors. Why in fact the ABP did such a thing is unclear, and probably so lost in the mists of history that it can only be speculated on. At least one explanation has been offered.[2] That is not my purpose. The fifth year has resulted in a number of serious dilemmas, not the least of which is its funding; at least one commentator indicated that it would result in an outlay of an additional $20 million per year by institutions sponsoring pathology education programs.[3] The costs quoted in Colloton's[3] presentation included resident stipends and fringe benefits, that portion of pathology department faculty costs attributable to clinical supervision of resident and administration of the residency program, as well as other expenses for staff support, supplies, and overhead.

Pathology practice and research is complicated, and getting more so. It is probably a truism that even before the fifth-year rule most pathology residents were spending 5 years in training; additionally, a number of surveys indicate that most residents currently recognize the need to possess a specialty, a "hobby," expertise, etc, in addition to broad AP/CP training, regardless of whether they enter private practice or academia.

The requirements of the ABP for the fifth certifying year are stated in the following manner: ". . . and one additional full credential year, which may be spent in further training or research in pathology or closely allied disciplines; in the practice of pathology; or in approved graduate medical education in internal medicine, surgery, obstetrics and gynecology, pediatrics, or family practice."[1] Certainly, a fifth year spent in the equivalent of a hematopathology (transfusion medicine, microbiology, etc) fellowship would qualify. But such education could not be claimed as credit toward the ABP certifying examination in that special or added qualification. To sit for these examinations, the resident must spend another year (two for some areas such as neuropathology) in fellowship training. One proposed solution for this state is the suggestion that the ABP grant credit toward the subspecialty certification examination if that training, even if it occurs during the fifth year, is acquired in an approved program. This was one of the unanimous recommendations of the 1989 ASCP (American Society of Clinical Pathologists) Colorado Springs (Colo) Conference, entitled "The Future Content and Structure of Residency Training in Pathology."[4] Since the fifth year would *also* serve as the certifying year, in effect this becomes a form of "double-dipping," or the awarding of dual credit for a single educational experience. This is not an unheard-of situation (MD/PhD candidates do this regularly), but it is not common, nor generally approved of. Thus even if the members of the

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ABP were to vote to award such dual credit, it is highly unlikely that the American Board of Medical Specialties, to whom they are accountable, would allow them to do such a thing. Thus, the probability that this rule will be changed, or could be changed, is slim. In order to gain the requisite expertise as pathologist-subspecialists, to claim such expertise for themselves, and to pursue a practice in which that subspecialty will constitute a sizable portion of their practice, do residents in fact need to spend yet another year in fellowship education? Not necessarily so. The purpose of this article is to explain why this is the case.

Bear in mind, the ABP, unlike almost all of the other 23 specialty boards, grants credit toward subspecialty certification *on the basis of appropriate practice,* as well as for the completion of formal fellowship training. Generally, 2 years of practice are equivalent to 1 year of formal training. Remember this as you read the following pathology career educational vignette.

> Dr X graduates from University A, then enters an accredited AP/CP program at University B, where she completes the 3-year core, does a fourth year of electives that she and her director decide on, then subsequently spends her fifth year in Dr Y's laboratory doing the equivalent of a hematopathology fellowship. Since she was a fine resident/fellow, Dr Y is perfectly willing to write a letter stating that she is competent to begin practice as an entry-level hematopathologist. She may even have published several papers, in which case he is willing to say that she also has definite academic potential. He will probably try to keep her as his associate director. Dr X, however, enters practice with a seven-person group as a hematopathologist expert, and in the year following her 5-year residency (her first year of practice) she takes the AC/CP Board examination, claiming the hematopathology year as the certifying year. She passes. Two years later (her third year of practice), she takes the hematopathology examination, claiming the 2 years of practice as equivalent to 1 year of hematopathology fellowship. She also passes that examination, receives a certificate, and hangs it next to her AC/CP certificate.

Can she do this? Is it appropriate? Of course. Everyone knows where Dr X received the education that enabled her to assume her postresidency position. The letter from Dr Y and the University B certificate of completion are much more important than the ABP certificate in this regard. She had to delay for 2 years the gratification of seeing the hematopathology certificate hanging in her office, but she was probably better prepared for the actual examination when she took it. Not many residents are likely to take the basic certifying and specialty examinations during the same year. The fifth, certifying year has in this case been used to gain subspecialty expertise, and is probably responsible for her first job. It has also served as the certifying year. The subspecialty certificate will come in a few years, probably more easily. The resident is happy. No dual credit has been awarded. The American Board of Medical Specialties is happy. The application for subspecialty certifying examination was made after an appropriate practice experience. The ABP is happy.

Of course not everyone is happy because many still oppose the original institution of the fifth-year rule. We have a surfeit of problems in pathology, all awaiting our attention. With the Residency Review Committee for Pathology, ABP, and Park City recommendations for pathology residency education now finally aligned, this may be the time to let the fifth-year argument be. Let it be, and let us begin to concern ourselves with matters we may possibly be able to do something about, such as financing the fifth year and restructuring our residencies so as to educate pathologists who will practice, teach, and carry out investigations in the 21st century.

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Adenomatoid Tumor of the Adrenal Gland With Ultrastructural and Immunohistochemical Demonstration of a Mesothelial Origin

William D. Travis, MD; Ernest E. Lack, MD; Norio Azumi, MD; Maria Tsokos, MD; Jeffrey Norton, MD

• We describe an adenomatoid tumor arising in the left adrenal gland of a patient who underwent bilateral adrenalectomy for the ectopic adrenocorticotropic hormone syndrome. The mesothelial origin of the tumor was confirmed by electron microscopy and positive immunohistochemical staining for keratin. The tumor had a prominent cystic component raising the question whether adrenal cysts are derived from mesothelial inclusions. It is proposed that consideration be given to adding a new category of "mesothelial cysts" to the classification of adrenal gland cysts.

(*Arch Pathol Lab Med.* 1990;114:722-724)

Adenomatoid tumors are relatively uncommon tumors that characteristically arise in the male and female genital tract, particularly the epididymis, but have also been encountered in the spermatic cord, tunica albuginea of the testis, prostate, and ejaculatory duct.[1,2] In females these tumors have been most commonly seen in the uterus and fallopian tube and rarely in the ovary.[3] Adenomatoid tumors have also been reported in the omentum, retroperitoneum, the adrenal gland, and in the periadrenal region, but these cases are extremely rare.[3-5] The occurrence of adenomatoid tumors actually within the substance of the adrenal gland has been briefly mentioned but not illustrated.[3,6] Several of the cases reported by Plaut[7] as locally invasive adrenal lymphangiomas appear histologically identical to adenomatoid tumors. An adenomatoid tumor, associated with an adrenal cortical rest in the spermatic cord has also been described.[8] To the best of our knowledge, however, an intra-adrenal adenomatoid tumor has not been reported with ultrastructural and immunohistochemical documentation of a mesothelial origin.

For editorial comment, see p 641.

REPORT OF A CASE

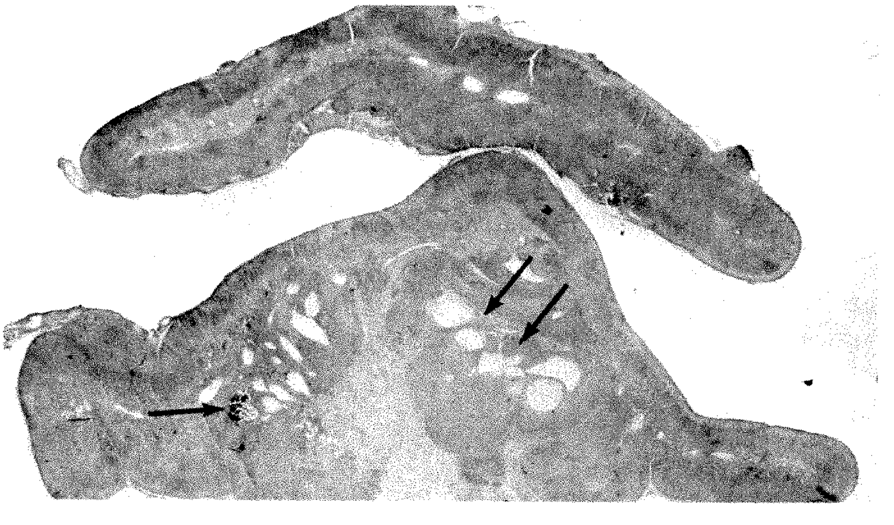
A 24-year-old man presented with muscle and bone pain and was found to have Cushing's syndrome. He was subsequently shown to have the ectopic adrenocorticotropic hormone syndrome due to an atypical bronchial carcinoid tumor. Seven months later bilateral adrenalectomy was performed to control severe manifestations of Cushing's syndrome. Preoperative radiographs demonstrated a left unilateral adrenal nodule measuring approximately 1.0 cm. The patient later developed numerous sclerotic metastases to long bones, and a computed tomographic scan revealed multiple pulmonary metastases that on open lung biopsy proved to be metastatic carcinoid tumor. Six months later the patient died with massive pulmonary metastases.

PATHOLOGIC FINDINGS

The right and left adrenal glands weighed 6 and 10 g, respectively, the right adrenal gland measured 6 × 4 × 1 cm, and the left adrenal gland measured 6 × 4 × 2 cm. The adrenal cortex on both sides was diffusely hyperplastic measuring 3 mm in thickness. The left adrenal gland contained an ill-circumscribed, 1.1-cm pale-white nodule with a peripheral rim of yellow tissue.

Histologically, the nodular lesion in the left adrenal gland was unencapsulated with ill-defined extension into adjacent cortex and fingerlike extensions separating cords and nests of cortical cells (Fig 1). There was also extension into the periadrenal adipose tissue in the hilar region of the gland. At the periph-

Fig 1.—Low-power view of adrenal cross section shows an ill-circumscribed nodule at the base of the gland. The tumor extends into the periadrenal fat and at the periphery shows a prominent multicystic component (double arrows). The surrounding adrenal cortex is hyperplastic with conversion of the lipid-rich cells of the zona fasciculata to lipid-poor, eosinophilic compact cells. A focus of myelolipomatous metaplasia is present at the edge of the tumor (single arrow) (hematoxylin-eosin, × 4).



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ery, there were prominent cystlike spaces. The tumor was composed of nests and cords of uniform, epithelioid cells arranged primarily in a cordlike pattern. This seemingly solid cordlike architecture had distinctive adenoid or glandlike features on close inspection with coalescence of intracytoplasmic vacuoles. Single cells occasionally had eccentrically placed nuclei with a cytoplasmic vacuole giving a signet ring appearance (Fig 2). The nuclei were uniform and round to oval with vesicular, pale-staining nuclei often with a single small nucleolus. Most of the cells lining the tubular spaces were flattened or cuboidal. Single cells occasionally had eccentrically placed nuclei and ~~a~~ cytoplasmic vacuole giving a signet ring appearance. Due to its infiltrative borders, small nests of adrenal cortical cells were present within the tumor. The tumor stained negatively for mucin and glycogen (periodic acid–Schiff with and without diastase); however, there was positive periodic acid–Schiff staining in the thin layer of connective tissue beneath the cells lining cystic parts of the tumor and focally outlining cords of tumor cells. The borders of the tumor cells stained for alcian blue, but no intracytoplasmic staining was found either before or after hyaluronidase digestion.

Histologically the ipsilateral adrenal cortex surrounding the tumor and in the contralateral gland demonstrated diffuse hyperplasia with conversion of lipid-rich zona fasciculata cells to lipid-poor eosinophilic, compact cells. A small focus of myelolipomatous metaplasia was present at the edge of the adenomatoid tumor.

Tissue from the tumor was removed from the paraffin block, deparaffinized in xylene, fixed in 2.5% glutaraldehyde solution, postfixed in osmium tetroxide, and embedded in Maraglass 655 (Polysciences). Thin sections from representative areas were examined with an electron microscope (Philips 201). Ultrastructurally, the tumor cells exhibited well-developed desmosomes, tortuous (bushy) microvilli on the outer surface, and a moderate amount of tonofilaments in the cytoplasm (Fig 3).

Immunohistochemical investigation was performed using the avidin-biotin-complex method and alkaline phosphatase with fast red as the chromogen. The tumor cells were strongly positive for keratin (AE1/AE3, Boehringer Mannheim Biochemistry, Indianapolis, Ind), weakly positive for epithelial membrane antigen (Dako Corp, Santa Barbara, Calif), strongly positive for vimentin (Dako Corp), negative for chromogranin (Dako Corp), and negative for corticotropin (Dako Corp). The keratin stain highlighted the tendril-like extension of the tumor into the adjacent adrenal cortex (Fig 4). The bronchial carcinoid tumor stained positively for corticotropin (Dako Corp).

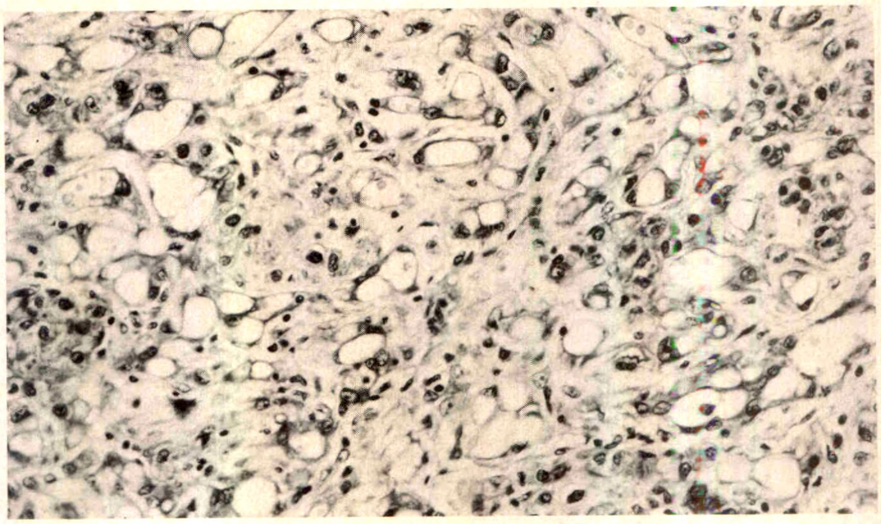

Fig 2.—The tumor is composed of nests and cords of epithelioid cells with characteristic glandlike or adenoid features. The tumor cells have large intracytoplasmic vacuoles and eccentrically placed nuclei (hematoxylin-eosin, ×250).

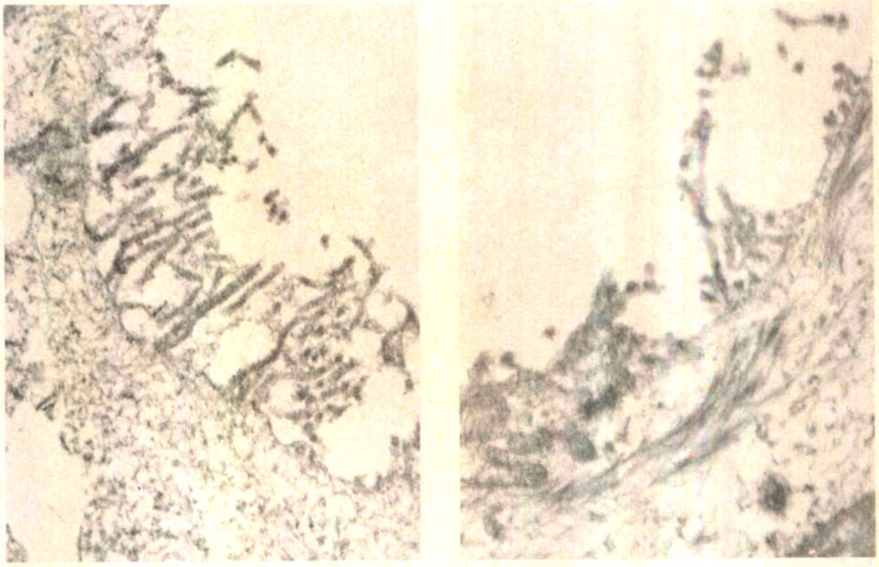

Fig 3.—Left, This electron micrograph demonstrates tortuous microvilli that were found along the luminal surface of the tumor cells (×18 200). Right, The cytoplasm of the tumor cells contains a moderate amount of tonofilaments (×18 200).

COMMENT

The occurrence of adenomatoid tumors of the adrenal gland raises interesting histogenetic and differential diagnostic considerations. Although extraordinarily rare, it is plausible that these tumors might arise in the adrenal gland, since there is a close embryologic relationship between the developing adrenal glands and the gonads[9] and these tumors are found most commonly in the genital tract of males and females. Heterotopic adrenal cortical tissue can be found in the periovarian and peritesticular regions, and rarely gonadal-stromal tissues have been reported in the adrenal gland in the form of Leydig cell and granulosa cell tumors.[9,10] These developmental relationships provide an embryologic basis for the occurrence of adenomatoid tumors in the adrenal gland.

We have demonstrated an adenomatoid tumor in the adrenal gland and confirmed its mesothelial origin with

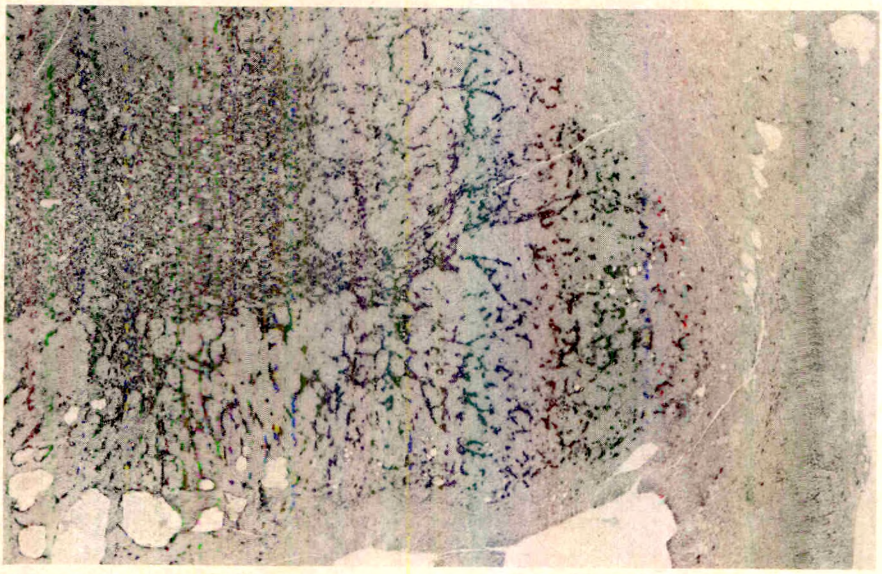

Fig 4.—The positive keratin staining of the tumor highlights its infiltrative borders with tendrils of tumor extending among the surrounding adrenal cortical cells. The cells lining the cystic areas at the periphery of the tumor also stain positively (keratin, ×11.5).

ultrastructural and immunohistochemical studies. The presence of long bushy microvilli and cytoplasmic keratin tonofilaments by electron microscopy in combination with the positive immunohistochemical staining for keratin, epithelial membrane antigen, and vimentin supports a mesothelial derivation, in keeping with previous studies of adenomatoid tumors found in more conventional locations.[11-14] In this particular case with the unusual setting of ectopic adrenocorticotropic hormone syndrome, the negative staining for chromogranin, as well as the light-microscopic features, point against a pheochromocytoma or metastatic carcinoid tumor. The negative staining for factor VIII supports the impression that it was not derived from vascular endothelium. The ultrastructural observations also exclude an endocrine or vascular origin of this adenomatoid tumor.

The occurrence of rare adenomatoid tumors in the substance of the adrenal gland or periadrenal tissues brings up the general topic of histogenesis of adrenal cysts.[15,16] Recently, an adrenal cyst with a lining that stained positively for keratin was reported.[6] Although this case was reported as a "true" epithelial cyst, a fundamental question was raised whether it could be mesothelial in origin. Since both types of cells are keratin positive, the issue of whether the cyst was epithelial vs mesothelial was not further clarified.[6] The demonstration in this case of the mesothelial origin of an adrenal adenomatoid tumor with a prominent cystic component suggests that a subset of adrenal cysts may actually be mesothelial rather than epithelial in origin. This concept is compatible with recent evidence suggesting that cysts of the spleen may be derived from proliferation of mesothelial inclusions.[17] Thus, mesothelial inclusions may provide an explanation for the existence of both adenomatoid tumors and mesothelial cysts in the adrenal gland.

It should be noted, as in this case, that adenomatoid tumors may have infiltrative borders. But this feature should not lead to the mistaken impression of a malignant tumor. Since adenomatoid tumors stain with keratin, a case involving the adrenal gland should not be mistaken for metastatic carcinoma, a difficult differential diagnostic problem in a patient with a known signet ring adenocarcinoma. However, adenomatoid tumors do not produce mucin and this can easily be determined with the periodic acid–Schiff, alcian blue, or mucicarmine stains.

The documentation in this case of a partially cystic adenomatoid tumor within the substance of the adrenal gland has important implications, on histogenetic and embryologic grounds, for the classification of "adrenal cysts." Specifically, some adrenal cysts previously believed to be epithelial may actually be mesothelial in origin. With these observations in mind, consideration should be given to adding a new category of "mesothelial cysts" to the classification of adrenal cysts. Further delineation of this proposed category of mesothelial adrenal cysts awaits immunohistochemical and ultrastructural documentation.

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Adenomatoid Tumor of the Adrenal Gland

Preston R. Simpson, MD

• An adenomatoid tumor of the adrenal gland is presented. The tumor was discovered in a patient with hypertension and was clinically mistaken for a pheochromocytoma. This is the first reported case, to my knowledge, of an adenomatoid tumor within the adrenal gland, with the exception of one previous article in which the lesion was mistaken for a lymphangioma. This case illustrates that adenomatoid tumors can rarely occur in extragenital locations, even in regions presumably devoid of mesothelial cells.

(*Arch Pathol Lab Med.* 1990;114:725-727)

Adenomatoid tumors are uncommon neoplasms that usually occur in the genital tract. Their occurrence in organs other than the epididymis, the uterus, or the fallopian tube is exceedingly rare. A case of an adenomatoid tumor of the adrenal gland is described.

For editorial comment, see p 641

This is the first reported case, to my knowledge, of such a tumor although previous cases may have been reported, but were not recognized as such by the authors at the time.

REPORT OF A CASE

A 44-year-old Hispanic man who was otherwise healthy presented with an episode of syncope. He was hospitalized and found to have a blood pressure of 270/180 mm Hg. A 24-hour urine collection revealed excretion of 60.6 μmol/d of vanillylmandelic acid (normal, <50.5 μmol/d). The assay for quantitative metanephrines could not be performed due to the presence of an interfering substance. A computed tomographic scan revealed a left adrenal gland tumor. The patient was taken to the operating room with a presumptive diagnosis of a left adrenal gland pheochromocytoma. A left adrenalectomy was performed. The operation and the postoperative course were uneventful.

PATHOLOGICAL FINDINGS

The adrenalectomy specimen measured 3.5 × 3.2 × 3.0 cm. At one end grossly normal adrenal cortex and medulla were visible. The cut surface showed a thin rim of cortical tissue in some areas. Much of the gland was replaced by a fleshy gray tumor with foci of what was interpreted as cystic degeneration. Focal calcification was present. A rim of normal adipose tissue was present over one surface (Fig 1).

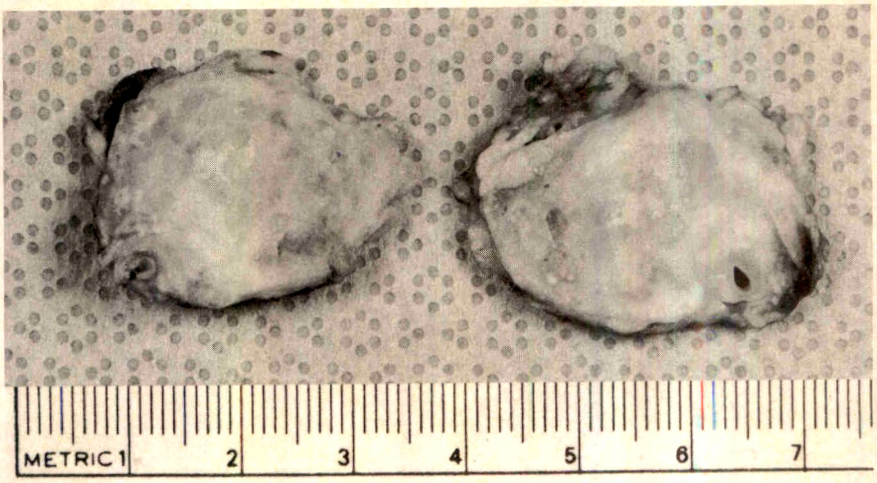
Microscopic examination showed subtotal replacement of the adrenal cortex and medulla by a neoplasm composed of round to oval tubular spaces with occasional branches. The spaces were lined by flattened cells, with scant to moderate amounts of eosinophilic cytoplasm (Fig 2). The tumor infiltrated the adrenal parenchyma, occasionally enveloping islands of residual adrenal cortex (Fig 3). Rare cells had a suggestion of a brush border when examined at high power (Fig 4). The nuclei were uniform and oval with many of them containing a single nucleolus. Nuclear atypia and mitotic activity were lacking. Occasional cells had clear cytoplasmic vacuoles. The tubular lumens contained small-sized and intermediate-sized lymphocytes in some areas. The stroma contained spindle-shaped fibroblasts with delicate collagen fibers and occasional foamy macrophages. Scattered aggregates of small lymphocytes were seen within the tumor and at the periphery (Fig 5). Sclerosis and dystrophic calcification were seen focally.

Mucicarmine and periodic acid–Schiff stains, with and without diastase digestion, revealed no evidence of glycogen or mucin content. Immunoperoxidase stains with antihuman cytokeratin (Becton Dickinson, Mountain View, Calif) were positive in the tumor cells, but negative for carcinoembryonic antigen (Dako Corp, Carpinteria, Calif).

COMMENT

Most adenomatoid tumors occur in the genital tract, specifically in the epididymis, fallopian tube, and uterine corpus[1,2] but they have occasionally been reported in the perirenal tissues,[3] the small-bowel mesentery,[4] and the omentum.[5] Most pathologists are familiar

Fig 1.—The tumor was a mostly solid mass with focal calcification and tiny punctate cysts.



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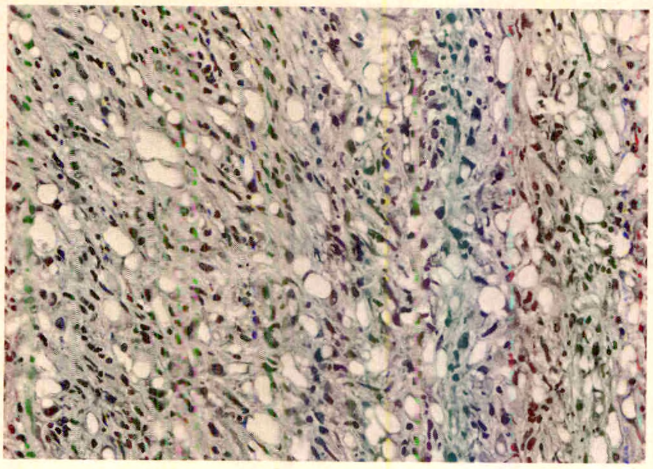

Fig 2.—The tumor consisted mostly of small tubules lined by flattened mesothelial cells (hematoxylin-eosin, ×250).

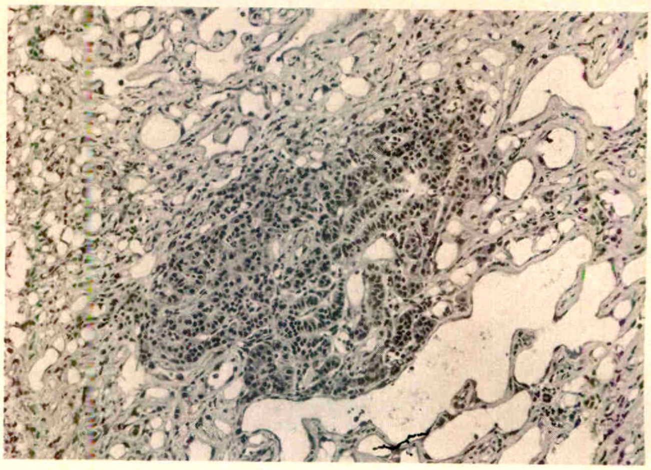

Fig 3.—The tubules infiltrate the adrenal gland and surround remnants of adrenal cortex (hematoxylin-eosin, ×125).

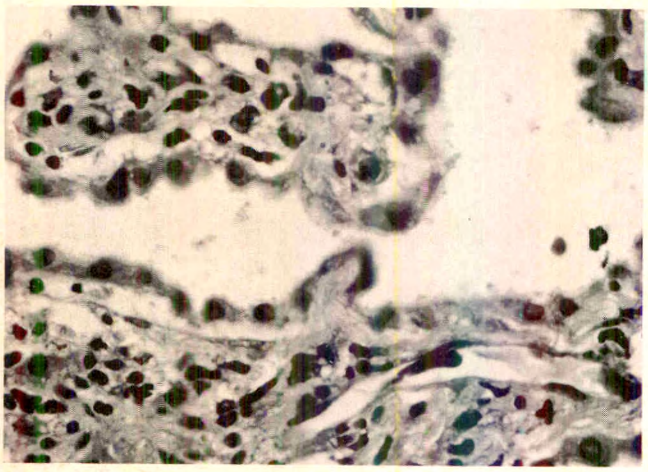

Fig 4.—In some areas the cells lining the tubules demonstrate a brush border (hematoxylin-eosin, ×600).

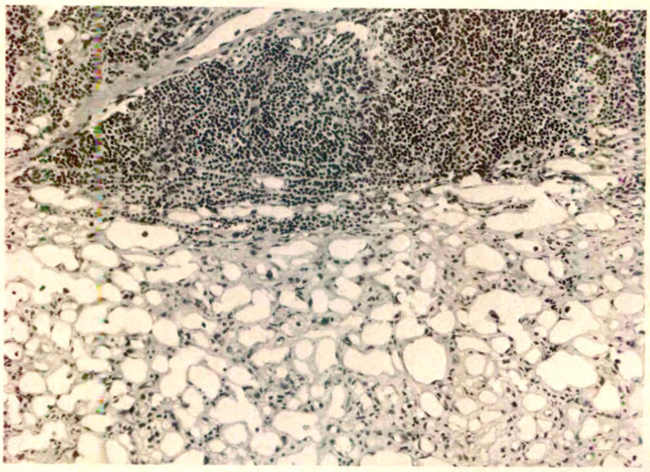

Fig 5.—Aggregates of small lymphocytes are present in various areas, particularly at the periphery of the tumor (hematoxylin-eosin, ×125).

with adenomatoid tumors in the genital tract, and the diagnosis does not present a problem. However, in the rare instances in which it occurs in an extragenital location, the diagnosis can be missed.[4]

The adenomatoid tumor is now generally accepted as being of mesothelial differentiation based on ultrastructural,[6-8] histochemical,[8] and immunoperoxidase[9] studies, although some[10] would argue that occasional ones can be endothelial. The reason for this peculiar mesothelial tumor being limited in its distribution is unknown.

This case is interesting not only for the rare extragenital location of the adenomatoid tumor but also for the occurrence in an organ where one would not expect a mesothelial tumor to arise. To my knowledge, this is the first case of an adenomatoid tumor within the adrenal gland. Evans et al[3] recently described a "suprarenal" adenomatoid tumor that had the adrenal gland stretched over one pole of the tumor, with microscopic examination showing adrenal cortex separated from the tumor by a thin fibrous layer. The mesothelial nature of that tumor was supported by ultrastructural findings.

Plaut[11] described four cases of "locally invasive lymphangioma" of the adrenal gland, but the gross and microscopic descriptions, as well as the photomicrographs, are more in keeping with adenomatoid tumors than lymphangiomas. The likelihood that Plaut's[11] tumors were adenomatoid has been pointed out previously.[4] Thus, this lesion has been previously reported but not recognized as such.

The presence of adenomatoid tumors in the adrenal gland is probably best explained by an origin from mesothelial inclusions that give rise to the tumors. This is the same mechanism that has been proposed for the rare true cysts of the adrenal gland.[12] Pathologists should be aware of the rare presence of mesothelial tumors in uncharacteristic locations to avoid misclassifying them as adenocarcinomas or as lymphatic tumors.

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Fluoxetine and the Bleeding Time

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• Fluoxetine (Prozac) is a nontricyclic serotonin (5-hydroxytryptamine) reuptake inhibitor commonly prescribed for the treatment of depression. Fluoxetine also blocks 5-hydroxytryptamine reuptake in platelets and could potentially lead to clinically significant platelet dysfunction. We describe a patient who developed petechiae and prolongation of the bleeding time while receiving fluoxetine therapy.

(*Arch Pathol Lab Med.* 1990;114:727-728)

Fluoxetine (Prozac), a nontricyclic serotonin (5-hydroxytryptamine) reuptake inhibitor, was approved in 1988 for the treatment of depression and has recently been found to be efficacious in the treatment of obesity[1] and fibrositis.[2] The most frequent side effects include nausea, nervousness, and insomnia and only very rarely are hematologic complications encountered.[3] The relative selectivity of this drug for inhibition of serotonin reuptake gives it great utility in the treatment of psychiatric disorders. In addition to actions in the central nervous system, fluoxetine also blocks 5-hydroxytryptamine reuptake in platelets and thus could deplete or reduce platelet serotonin stores.[4] Nevertheless, prolongation of the bleeding time, the best available in vivo test of platelet function, was observed in only one of 6724 patients receiving fluoxetine in clinical trials and none suffered hemorrhagic complications (Lilly Research Laboratories, unpublished data, 1989). We present a case in which the occurrence of petechiae and prolongation of the bleeding time were attributable to fluoxetine therapy.

REPORT OF A CASE

A 44-year-old postmenopausal woman was referred to the University of Virginia Hematology Clinic, Charlottesville, for evaluation of petechiae and prolongation of the bleeding time (>15 minutes). She had noted mild easy bruising her entire life, with the development of a large ecchymosis following trauma 20 years ago. The patient had suffered recurrent unilateral epistaxis 10 years earlier, which was responsive to cauterization without recurrence. Over the past 2 years she had noted persistent red pinpoint spots on her inner arms bilaterally and intermittently on her shins. She denied other symptoms of disordered hemostasis. Her only previous surgery included a tubal ligation 15 years ago and a salivary duct stone removal 1 year ago, both without hemorrhagic complications. She had been treated for hypertension for 5 years with hydrochlorothiazide, 25 mg/d, and nadolol, 80 mg/d. At no time did the patient receive estrogen replacement therapy. For the past 2 years she had been taking 20 mg of fluoxetine every other day for symptoms of depression. She denied alcohol and cigarette use and her family history was noncontributory. Examination was notable for numerous petechiae on her inner arms bilaterally, but nowhere else. Results of a laboratory evaluation showed a normal platelet count (289 × 10^9/L) and normal platelet morphology. Her prothrombin time and activated partial thromboplastin time were normal, with a bleeding time prolonged to 11.5 minutes (normal, 1.0 to 7.5 minutes). Assays of von Willebrand factor showed the following normal results: factor VIII: C activity, 112 U/dL (normal, 45 to 145 U/dL); ristocetin cofactor activity, 108% (normal, 47% to 196 %); and von Willebrand factor antigen, 135% (normal, 49% to 192%). The fluoxetine was discontinued for 2 weeks and the patient was restudied. Her petechiae had decreased in number and the bleeding time was normal (6.5 minutes). Results of platelet aggregation studies, using platelet-rich plasma with adenosine diphosphate, epinephrine, ristocetin, collagen, and serotonin as agonists, were normal. The patient resumed fluoxetine treatment for relief of symptoms of depression and was seen again 2 weeks later. Her petechiae had returned and her bleeding time was again prolonged (>15 minutes). A repeated ristocetin cofactor activity assay was again normal (140%). She subsequently was able to discontinue the fluoxetine and within 1 month her petechiae had completely resolved.

COMMENT

Bleeding disorders from platelet dysfunction following therapy with nontricyclic 5-hydroxytryptamine reuptake inhibitors are rare. Those patients who develop platelet dysfunction in response to fluoxetine may either have a mild underlying platelet disorder that is exaggerated by depletion of dense gran-

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Fluoxetine Therapy—Humphries et al 727

ule serotonin or have an altered platelet 5-hydroxytryptamine reuptake system. Given the previous evidence for multiple inhibitor-binding sites in the 5-hydroxytryptamine reuptake system,[5] an abnormal binding site for nontricyclic drugs could modify the potency of these compounds without directly affecting 5-hydroxytryptamine binding or reuptake function under drug-free conditions. The absence of a detectable platelet disorder in our case when the patient was drug free is consistent with this speculative mechanism. In view of the long half-life of this medication (1 to 3 days) and its active metabolite (7 to 15 days),[4] some caution should be exercised when treating patients with thrombocytopenia, or with proven or suspected platelet dysfunction.

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Small-cell Carcinoma of the Vagina

A Clinicopathologic Study

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• We report a case of small-cell carcinoma of the vagina in a woman whose entire uterus, cervix, both fallopian tubes, and both ovaries had been removed 22 years previously. She presented with diffuse submucosal disease of the distal vagina with a possible soft-tissue component extending beyond the end of the vaginal vault. Extensive microscopic and immunohistochemical assessment established the diagnosis; treatment was initiated with chemotherapy, followed by local radiotherapy. To our knowledge, this is the first fully characterized case of its kind. We discuss the aggressive local behavior and the characteristics of gynecologic small-cell tumors in general.

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Extrapulmonary small-cell carcinomas are rare, constituting only 4% of small-cell tumors in a previous study.[1] These tumors can arise from various organ systems, including the gynecologic tract.[2-8] Small-cell or neuroendocrine carcinoma of the cervix has been described recently as a distinct clinicopathologic entity.[9] Unlike earlier studies, a recent study by Barrett et al[9] used special stains and electron microscopy (EM) in addition to routine histologic studies to characterize these tumors. The identification of neurosecretory granules is the most definitive evidence of small-cell carcinomas, and EM appears more sensitive than argyrophilic staining in detecting these granules.[10,11]

We report herein a case of small-cell carcinoma of the vagina. To our knowledge, only one other case has been mentioned in the literature and that case was not singled out for characterization among a series of cervical small-cell tumors.[12] The absence of a uterus, cervix, and both fallopian tubes and ovaries due to surgery 22 years earlier makes the origin of this tumor in another part of the gynecologic system unlikely.

REPORT OF A CASE

Clinical Findings

A 63-year-old white woman presented with a 1-month history of intermittent vaginal bleeding. A total abdominal hysterectomy and right salpingo-oophorectomy had been performed at age 39 years for dysfunctional uterine bleeding. Two years later, the left adnexa were removed for an ovarian mass; a benign ovarian cyst was found. Physical examination at presentation revealed a small soft pink–tan nodule, 2 cm in diameter, in the right upper anterior portion of the vagina. A biopsy was performed and the results were reported as small-cell carcinoma.

On physical examination at our center 3 weeks later she was found to have nodularity at the biopsy site and two new nodules adjacent to this site measuring 3 and 5 mm^2 in area. No paravaginal masses were seen. Cystoscopic examination revealed a normal mu-

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cosa and slight elevation in the trigone area that corresponded to the area of the vaginal lesions. Biopsy specimens, which were obtained from both new nodules, were diagnosed as small-cell carcinoma. The patient underwent staging investigations. Chest roentgenogram and computed tomographic (CT) scan of the thorax were normal. A CT scan of the abdomen and pelvis suggested a small focal collection of soft tissue just posterior to the bladder and at the distal vaginal level on the left side. No invasion of the bladder or rectosigmoid was noted. An ultrasound scan of the pelvis done within 1 week was normal. It was concluded that this might represent external growth of the tumor from the vagina but it was not arising from urinary bladder or rectosigmoid.

Following staging tests and 2 weeks after the second biopsy, erythema, edema, and easily induced local bleeding were noted at the biopsy sites. No bulky intravaginal or extravaginal disease could be palpated on bimanual pelvic examination. It was decided to treat the patient with combination chemotherapy because of the likelihood of microscopic metastatic disease with this histologic type. She was initially treated with etoposide (VP-16) and cisplatin (cis-platinum), one of the most effective combinations for the treatment of small-cell carcinomas. The management plan included radiotherapy to the vagina following six courses of chemotherapy.

The patient experienced severe neutropenia and fatigue following her first course of chemotherapy. Three weeks after chemotherapy was given, the vaginal mucosa was normal by inspection. By palpation, fine nodularity persisted around the sites of the previous biopsies. A second cycle of chemotherapy again resulted in severe neutropenia necessitating a delay in treatment. At reexamination 4 weeks after her second cycle, a 1.5 × 3.0-cm area of coarse nodularity was noted on palpation in the submucosal portion of the distal right anterior part of the vagina. The mucosa appeared normal. Repeated CT scan of the pelvis showed no pelvic masses or other abnormalities. One week later, mucosal disruption over the nodular area was noted. Because of this local disease progression, radiotherapy was initiated to encompass the pelvis and lower para-aortic regions.

She was well for 6 months before episodes of confusion and memory loss developed. On admission to the hospital, a full diagnostic workup concluded with a diagnosis of a syndrome of inappropriate antidiuretic hormone secretion. Complete examination and restaging tests, including CT scan of thorax, abdomen, and pelvis, failed to show recurrent or metastatic tumor. With fluid restriction and demeclocycline hydrochloride therapy, her clinical condition returned to normal and serum sodium levels rose to near normal values. She was discharged from the hospital on a regimen of demeclocycline and close clinic follow-up was planned.

Three weeks after hospital discharge, she was transferred to our hospital with a diagnosis of recurrent syndrome of inappropriate antidiuretic hormone secretion. At the initial assessment, the patient's platelet count was subnormal (168×10^9/mL). A bone marrow aspiration and biopsy were performed; the findings showed carcinoma cells consistent with recurrent anaplastic small-cell carcinoma. Again, no recurrent local pelvic tumor was found. She was transferred to another hospital for wide-field radiotherapy but she died within 6 weeks. Postmortem examination was not performed.

Materials and Methods

The tumor was scrutinized by light microscopy, EM, and immunohistochemical techniques. Studies with special stains for the demonstration of cytoplasmic granules, including Grimelius, Masson-Fontana, and Churukian stains were carried out. Immunohistochemical staining for neuron-specific enolase (NSE), cytokeratins, CAM 5.2 (Becton-Dickinson, Mountainview, Calif), and AE1/AE3 (Hybritech, San Diego, Calif) were also performed. Electron microscopy was used to detect the ultrastructural detail of cytoplasmic granules. Tumor specimens in formaldehyde solution–fixed paraffin-embedded blocks were deparaffinized and postfixed for 1 hour at 4°C in 1% cacodylate-buffered osmium tetroxide. Prepared specimens were examined using an electron microscope (Philips 301).

RESULTS

Because of its unique clinical presentation and the therapeutic implications, this case was carefully scrutinized as to its histologic type. Under light microscopy, the tumor showed a diffuse submucosal infiltrate of small cells with round to oval and spindle-shaped nuclei with scant cytoplasm. Hyperchromasia, individual cell necrosis, and a high mitotic rate were prominent features. Focally, islands and anastomosing trabeculae of cells separated by collagen tissue were noted. The overlying vaginal mucosa was completely normal (Fig 1) and composed of benign stratified squamous epithelium. At no point did the tumor encroach on this epithelium. There was no obvious continuity between the tumor and overlying squamous epithelium.

Studies with special stains for the demonstration of cytoplasmic granules (Grimelius, Masson-Fontana, and Churukian) were negative. Immunohistochemically, the tumor cells were focally but strongly positive for NSE as well as for cytokeratin and CAM 5.2.

Ultrastructurally, the cells were closely apposed but no obvious cell junctions were identified. The nuclei were round to oval with clumping and margination of chromatin. Distinct, neurosecretory granules were identified, measuring approximately 150 μm in diameter (Fig 2). These results confirmed the diagnosis of small-cell carcinoma.

COMMENT

Ulich et al[12] reported the most complete characterization of gynecologic small-cell tumors to date. Nine cervical tumors and one vaginal tumor were assessed by EM and a panel of immunohistochemical stains. The vaginal tumor was not described separately. While results using argyrophilic stains and immunohistochemical stains for serotonin, chromogranin, keratin, CAM 5.2, AE1/AE3, carcinoembryonic antigen, and S100 protein showed variable degrees of positivity, all tumors tested stained positively for NSE (10/10). Similarly, all specimens showed ultrastructural evidence of dense-core secretory granules.

In the series of Barrett et al,[9] three small-cell tumors of the cervix studied by EM displayed neurosecretory granules but none of these three stained for NSE and only one stained for neurosecretory granules by the Grimelius method. Neurosecretory granules were detected in some tumors with evidence of squamous or adenomatous differentiation. This supports other evidence that small-cell carcinomas may have a potential to differentiate along several morphologic lines. However, the definition of a neuroendocrine small-cell carcinoma requires the detection of neurosecretory granules by EM and morphologic criteria for small-cell carcinoma (sheets of small cells with trabeculae, rosettes, or ribbons).[9] A recent report of poorly differentiated neuroendocrine carcinoma of unknown primary required only the presence of dense-core granules on EM as definitive criteria for this entity.[13]

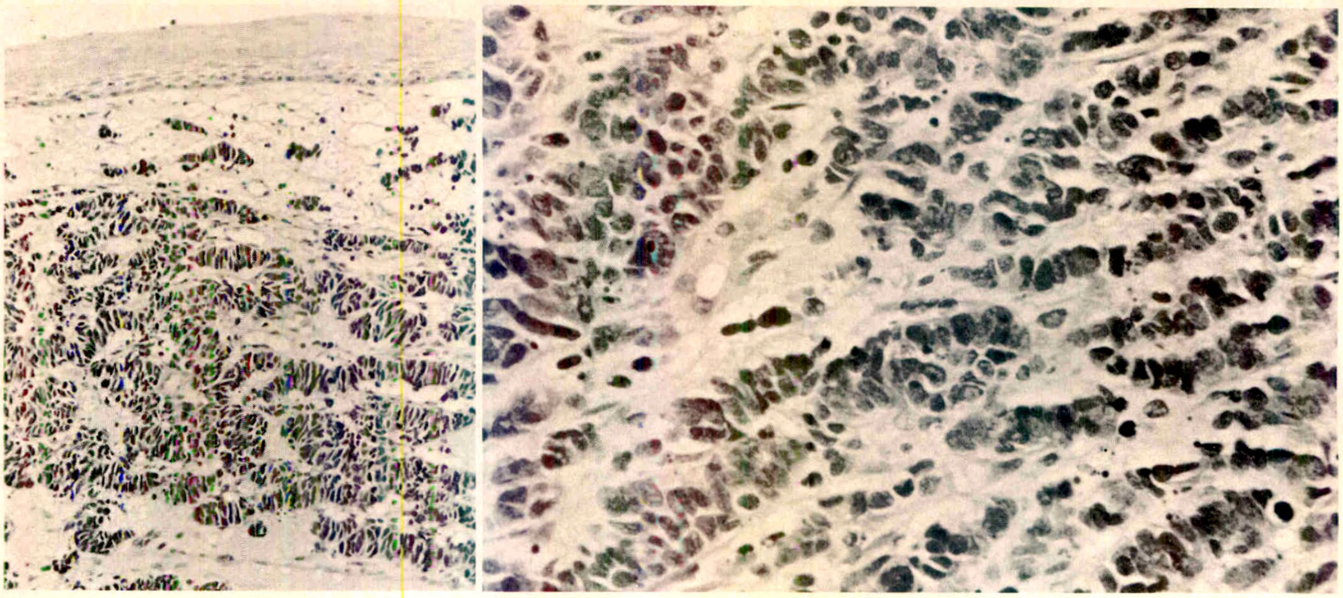

Fig 1.—Left, Small-cell anaplastic carcinoma involving the submucosa. The overlying epithelium is histologically normal (hematoxylin-eosin, ×30). Right, Small-cell anaplastic carcinoma of vagina. High-power view showing small hyperchromatic nuclei arranged in a trabecular pattern (hematoxylin-eosin, ×250).

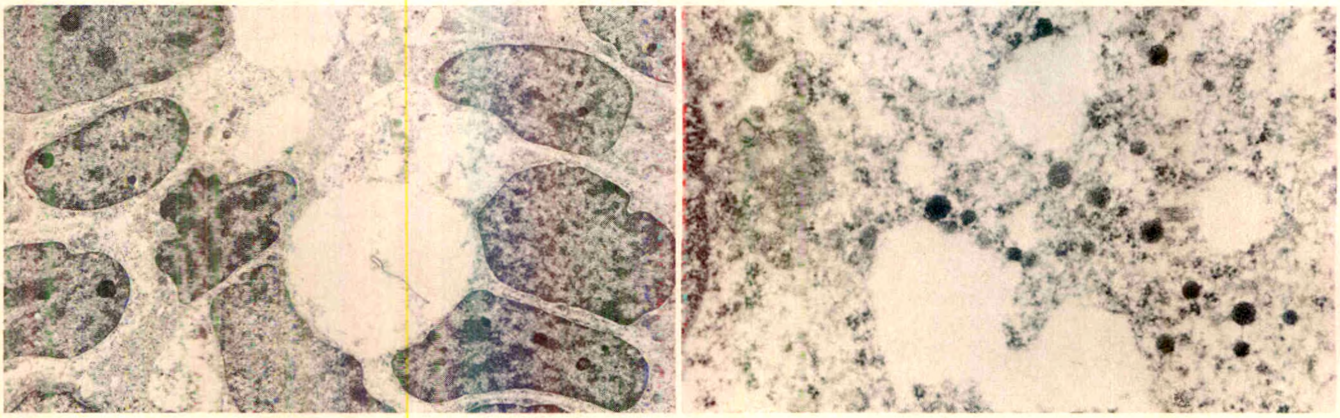

Fig 2.—Left, Low-power view of the ultrastructural features of small-cell anaplastic carcinoma of the vagina showing tightly packed cells with little cytoplasm (×5000). Right, High-power view showing distinct membrane-bound neurosecretory granules in the cytoplasm (×40 000).

Our case is morphologically typical of small-cell carcinomas that originate from the lung and other organs. Positive staining for NSE and characteristic neurosecretory granules on EM confirmed the diagnosis. Extensive staging investigations showed no evidence of a primary lung tumor, including repeated chest roentgenograms 3 months after the original biopsy. The small focus of soft tissue adjacent to the distal part of the vagina may have represented growth in the vaginal lesions or may have represented artifact. Cystoscopy and repeated CT scan provided no evidence for a primary tumor of the urinary bladder or rectosigmoid invading the vagina. Removal of the whole cervix 24 years earlier rules out the possibility of a primary cervical carcinoma.

Therapy for this tumor was determined by the clinical behavior of small-cell carcinomas from other sites.[1-9] Lung small-cell tumors rapidly metastasize when the primary tumors are small. Chemotherapy with cyclophosphamide, doxorubicin hydrochloride, and vincristine sulfate or etoposide and cisplatin results in excellent responses and up to a 15% 3-year rate of disease-free survival in patients with limited stage disease.[14-16] Appropriate therapy for small-cell tumors from other sites is less clear but most authors acknowledge a high propensity to metastatic progression and recommend chemotherapy as initial therapy.[1,2,9] While squamous or glandular elements are found in about 15% of small-cell carcinomas of the lung and could predict for a lower response rate, only cells consistent with small-cell carcinoma were found in the marrow.[17,18]

The development of the syndrome of inappropriate antidiuretic hormone secretion is intriguing because of its frequent association with small-cell carcinomas of the lung. The initial absence of detectable recurrent tumor suggested an unknown cause or the presence of a small volume of tumor that secretes a high level of ectopic antidiuretic hormone. The clinical suspicion remained high, however, and gross tumor was detectable in the bone marrow within several weeks. Retreatment was difficult in light of the previous poor tolerance and response to chemotherapy.

In 1982, small-cell carcinoma of the endometrium was reported by Olson et al[5] in a 60-year-old woman. The case of Ulich et al[12] involving the vagina was not discussed in detail and may have represented local metastatic disease spreading submucosally from the cervix. Our case clearly demonstrates the potential for rapid submucosal spread when the vagina is involved. A report by Barrett et al[9] suggests that cervical tumors have a poor prognosis similar to small-cell tumors of other sites, even when patients present with stage I disease. Their study and others suggest that the presence of neurosecretory granules may be associated with a poor outcome.[9,11] Whether the same is true for small-cell carcinomas originating in the vagina is yet unclear.

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Adenocarcinoma of the Uterine Cervix of Small Intestinal Type Containing Numerous Paneth Cells

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• A case of invasive adenocarcinoma of the uterine cervix with small intestinal differentiation to include numerous Paneth cells is presented. The light microscopic findings are confirmed by immunohistochemical and electron microscopic study. To our knowledge, this is only the second reported case of small intestinal cervical adenocarcinoma that contained Paneth cells and the first with ultrastructural and immunohistochemical confirmation. A small focus of in situ adenocarcinoma with similar features is seen adjacent to the invasive area.

(*Arch Pathol Lab Med.* 1990;114:731-733)

Adenocarcinomas of intestinal type that contain Paneth cells are seen most commonly in the gastrointestinal tract,[1] but have rarely been reported in the gallbladder,[2] urinary bladder,[3] sinonasal region,[4] and cervix.[5] We present the light and electron microscopic features of an invasive adenocarcinoma of the uterine cervix of small intestinal type that contained numerous Paneth cells.

REPORT OF A CASE

A 41-year-old-woman complained of a copious vaginal discharge and postcoital hemorrhage. Her cervix was described as enlarged, firm, and friable. A cytologic smear was diagnosed as adenocarcinoma. Her previous smear, 15 years earlier, was negative. A

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punch biopsy disclosed adenocarcinoma containing intestinal-type epithelium including goblet cells and cells with eosinophilic granules in the cytoplasm resembling Paneth cells. A radical hysterectomy was performed. Fifty-two pelvic and periaortic lymph nodes were removed without evidence of metastases. The ovaries were normal. The patient had no evidence of recurrent or metastatic disease 16 months after her surgery.

MATERIALS AND METHODS

Serial circumferential sections of the cervix were fixed in formaldehyde solution and embedded in paraffin. These were stained with hematoxylin-eosin. Special stains included Masson trichrome, phosphotungstic acid–hematoxylin, Fontana's silver impregnation, Sevier-Munger silver, periodic acid–Schiff, and mucicarmine. Immunohistochemical staining for lysozyme (1:300, Dako Corp, Santa Barbara, Calif) was performed on paraffin-embedded sections using the avidin-biotin-peroxidase complex technique.

Tissue for electron microscopic examination, previously fixed in formaldehyde solution, was postfixed in Karnovsky's phosphate buffer solution and subsequently embedded in Spurr's resin. Thin sections were cut on a microtome (Reichert Ultracut E), stained with uranyl acetate–lead citrate, and examined with a microscope (Zeiss 10 CA).

PATHOLOGIC FINDINGS

Gross Findings

No obvious tumor mass was seen. Instead, the cervical mucosa was replaced circumferentially by a yellow, mucoid-appearing tissue that extended 2.5 cm upward from the transformation zone and into the cervical wall to a maximal depth of 1.5 cm.

Microscopic Findings

Light microscopic examination disclosed an invasive, well-differentiated adenocarcinoma. Superficially, the glands were small and irregular, while in the deeper levels many of them were markedly distended by large amounts of pale-staining mucoid material. In these areas, the glands were focally devoid of lining cells. The tumor cells showed differentiation toward small intestinal epithelium. The predominant cell was tall and columnar, similar to the crypt cell of the small intestine. There was a moderate amount of apical cytoplasm. The nuclei were moderately enlarged, elongated, and coarsely granular, with chromatin clearing and small nucleoli. Mitoses were not abundant, but were easily found. These cells were interspersed with occasional goblet cells containing large, clear cytoplasmic vacuoles. Scattered throughout the malignant glands, particularly in the superficial areas, were neoplastic cells resembling Paneth cells. These cells contained closely packed, small, intensely eosinophilic cytoplasmic granules in a supranuclear location. The nuclei of these cells were basally oriented and difficult to distinguish from those of adjacent cells (Fig 1). Some of the glands were lined almost entirely by Paneth cells (Fig 2). Mucicarmine and periodic acid–Schiff stains confirmed the presence of intracytoplasmic mucin in the cytoplasm of the goblet cells, along the apical portion of the other cells and filling the lumina of the cystically dilated glands. The phosphotungstic acid–hematoxylin stain demonstrated the granules of the Paneth cells, as did Masson trichrome. However, the granules were best visualized by the hematoxylin-eosin stain. A Fontana silver stain for argentaffin cells was positive in rare cells at the base of the malignant glands. The Sevier-Munger silver stain for argyrophilia was negative. The immunohistochemical stain for lysozyme was strongly positive in the cytoplasm of many more tumor cells than appeared as Paneth cells by light microscopy, perhaps an indication of partial Paneth cell differentiation. A section of small intestine, run as a control, stained for lysozyme only in the Paneth cells at the base of the crypts.

In the endocervix, adjacent to the upper border of the invasive tumor, was a focus of in situ adenocarcinoma. The glands in this area communicated with the surface epithelium and were not distorted in shape. No stromal response was seen around them, and they were located adjacent to other glands higher in the endocervical canal, which appeared normal. The cells lining the area of adenocarcinoma in situ were similar to those in the invasive areas, including Paneth cells and goblet cells. There was no focus of non-neoplastic intestinal metaplasia seen.

Fig 1.—Invasive adenocarcinoma of the cervix. The glands are lined by columnar cells, occasional goblet cells, and Paneth cells with dark granular apical cytoplasm (arrows) (hematoxylin-eosin, ×450).

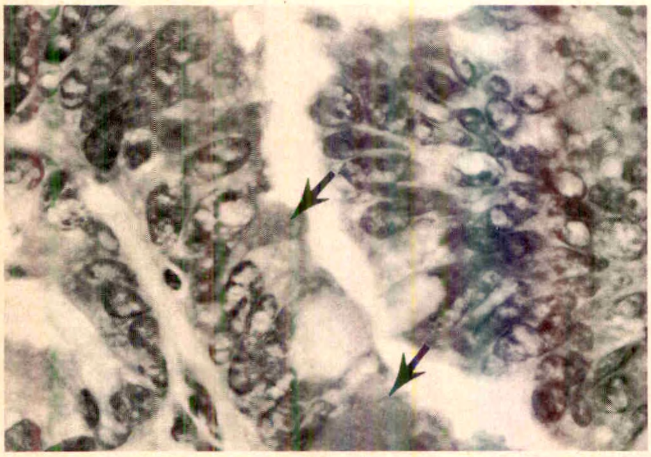
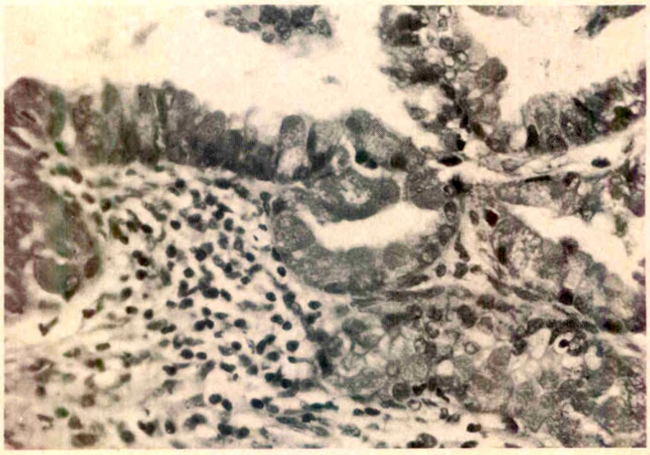


Fig 2.—Adenocarcinoma cells with abundant eosinophilic granular cytoplasm. Almost all of the epithelial cells in this field are neoplastic Paneth cells (hematoxylin-eosin, ×300).



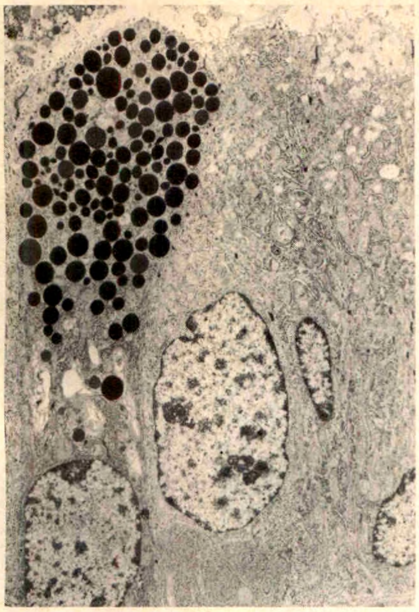


Fig 3.—Neoplastic Paneth cell with prominent suprabasalar electron-dense granules and basally oriented nucleus (×1600).

Ultrastructural Findings

The majority of neoplastic cells had the appearance of mucus-secreting epithelial cells. Junction complexes were prominent, and microvilli lined the apical surfaces where lumina were often formed by adjacent tumor cells. The microvilli projecting into the lumina had prominent filamentous core rootlets extending downward into the apical cytoplasm and were frequently associated with glycocaliceal bodies in the lumina. Scattered isolated epithelial cells contained large and very electron-dense granules. These cells had relatively sparse and stubby microvilli and a very prominent Golgi apparatus, typical of Paneth cells[6] (Fig 3).

COMMENT

Paneth cells, normally found at the base of the crypts of Lieberkühn in the small intestine of children and adults and in the colon of infants, still have an undefined function.[1] They are presumed to have a nutritional role because of their prominent Golgi structure and secretory granules. The presence of the bacteriolytic enzyme lysozyme and evidence of bacterial phagocytic activity in Paneth cells in rats suggest that these cells may play some role in controlling intestinal flora.[7] Paneth cells may be encountered in the colon of patients with chronic inflammatory processes such as ulcerative colitis and Crohn's disease and in gastric mucosa that has undergone intestinal metaplasia.[1,7]

Invasive malignancies outside the gastrointestinal tract containing small intestinal epithelial differentiation, which includes Paneth cells, are quite rare. These have previously been described in the gallbladder,[2] urinary bladder,[3] sinonasal mucosa,[4] and cervix.[5] Although small intestinal adenocarcinoma of the cervix containing goblet cells and argentaffin cells has been reported,[8] the single cervical cancer cited above is the only previously published example containing Paneth cells, to our knowledge. In that case, the tumor contained intestinal-type cells with brush borders, goblet cells, argentaffin cells, and "a few Paneth cells in a small proportion of the tumor acini." This is in contrast to the current case, in which Paneth cells were numerous and easily found. Also, confirmatory electron microscopic and immunohistochemical studies were not performed in the previous case.

Benign and premalignant small intestinal metaplasia of the cervix has been described. In one report,[9] cervical intestinal metaplasia was seen in three cases. In these three cases, however, there were no Paneth cells. Of interest is that in two of these cases there was evidence of concomitant squamous carcinoma in situ and what was thought to be "borderline glandular malignancy." Among 72 cases of adenocarcinoma in situ of the cervix,[10] 21 were thought to be of intestinal type (defined as areas where goblet cells were prevalent). In all 21 cases, there was also a component of ordinary endocervical in situ neoplastic change. In this group, argyrophil or argentaffin cells were found in four cases and Paneth cells were present in one.

In the present case, the finding of an area of in situ intestinal adenocarcinoma containing Paneth cells indicates that the invasive component of this very rare variety of cervical adenocarcinoma may have arisen from an area of small intestinal metaplasia containing Paneth cells that then proceeded to in situ and then invasive adenocarcinoma of a similar type. Alternatively, small intestinal differentiation of the neoplastic cells themselves, "neometaplasia," may have been the pathway of tumor cell differentiation. This possibility has been used to explain the müllerian origin of areas of intestinal appearance in benign and borderline mucinous ovarian neoplasms,[11] and might also apply to this cervical adenocarcinoma, presumably also of müllerian derivation. In either case, a small intestinal pattern of differentiation that includes Paneth cells, although extremely rare, must be included among the possible types of in situ and invasive adenocarcinomas of the cervix.

The authors thank Robert E. Scully, MD, for reviewing the case and Karen L. Brown for typing the manuscript.

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Image Cytometry

The Society for Analytical Cytology held its annual international meeting, March 18 through 23, 1990, in Asheville, NC. The Society is a group of engineers, research scientists, and physicians, all interested in quantitative cell analysis. This Society and its journal, *Cytometry,* are one of the prime sources of scientific information about quantitative cell analysis. This year, image cytometry, an alternate technique to the more popular flow cytometry, saw significant advances as a diagnostic tool for pathologists.

Image cytometers are quantitative microscopes that look at the image via a CCD solid-state camera. These cameras, much improved over the traditional television camera, are specifically designed for quantitative analysis of an image. The degree of resolution is measured by the number of "pixels" mapped per square micron of the image. A pixel is a single digitized element that is encoded as a number in computer memory corresponding to the light intensity from that element. The more pixels to an image, the more resolution. Theoretical considerations suggest that 100 pixels per square micron would produce an image equal to the limit of resolution of a light microscope. This degree of resolution has not yet been reached, but 16 pixels per square micron are common. This means that an epithelial cell on an endocervical smear, with a surface area of 100 square microns, is represented as a digitized image by some 1600 pixels.

Once the image has been digitized, the computer software goes to work. With each cell representing thousands of data points, a powerful computer is necessary. Desktop microcomputers, augmented by special image processing boards, are now up to the task. Various software applications allow automated recognition of cell types or quantification of cell parameters such as volume or nuclear shape. Alternatively, the software may operate in an interactive mode allowing the user to point to cells on the television screen and ask specific features to be measured.

An example of these applications is the measurement of DNA content in cells. This allows for DNA ploidy histograms on clinical samples such as cytologic smears, fine-needle aspiration samples, or paraffin-embedded histologic sections. The computer recognizes individual cells and discards artifacts or overlapping cells. The DNA in the cell is stained with a fluorescent dye such as propidium iodide. The relative intensity level of fluorescent light coming from the nuclear area is a measure of DNA content for that cell.

A common variation on this application is to project a low-power image on the screen of a computer work station. The pathologist selects any abnormal tumor cells from a background of stromal or inflammatory cells and asks for DNA analysis on this selected group only. The ability to select cells based on morphology is a distinct advantage of image cytometry compared with the companion technology of flow cytometry. Flow cytometry, although fast, requires single-cell suspensions and can look at only a few subpopulations of cells. Image cytometry can utilize traditional slide preparations and can separately analyze any group of cells the operator visually identifies.

In addition to the well-developed applications for DNA ploidy studies, image cytometry at this meeting was seen to be opening up in a number of new directions. Several groups at the Society for Analytical Cytology meeting reported general purpose digital microscope work stations. These advanced microscopes permit the user to interact with digital images for a large variety of purposes. One such application is the storing of images for reference purposes, replacing the traditional photographic slide collection. Digital images may be recorded on large scale storage devices, each image requiring up to 4 megabytes of space. Once digitized and saved as a file, an image can be processed by a number of general purpose software routines. It is now feasible for a pathologist to generate a report combining images with text. Additionally, consultation on cases may be obtained by connecting work stations via a network. The quality of digital images in these systems equals or exceeds the best images viewed through the oculars of a high-quality microscope. The reason the image exceeds the best optical microscope is color and contrast enhancement programs and other techniques for making the image "better than real."

The skills of a surgical pathologist are not supplanted by image cytometry but, in fact, the quantitative data appear to enhance the abilities of a morphologist to discern subtle distinctions. The near future holds the likely prospect of all surgical pathologists referring to their basic tool as a microscope work station, looking at screens rather than down the tubes of ocular eye pieces.

DENNIS W. ROSS, MD, PHD
Chapel Hill, NC

News and Comment

Molecular Biology Course Announced

The American Association of Pathologists will cosponsor, with the **US and Canadian Academy of Pathology,** a 3½-day course entitled "Concepts in Molecular Biology" from November 1 through 4, 1990, in Bethesda, Md. The course will consist of lectures, tutorials, laboratory demonstrations, and a syllabus with annotated bibliographies and laboratory protocols. The course has been designed for diagnostic and experimental pathologists, basic scientists, and clinical investigators who desire to become conversant with the basic principles and concepts of recent advances in biotechnology. Specifically, emphasis will be placed on understanding nucleic acid molecular biology and its application to diagnosis and pathogenesis of human disease. Because space for course attendance is limited, registrations are being accepted on a first-come, first-served basis. Category 1 continuing medical education credit will be available. For more information and registration forms, contact the American Association of Pathologists, 9650 Rockville Pike, Bethesda, MD 20814; (301) 530-7130.

Books

Section Editor: Robert D. Langdell, MD

Principles and Practice of Infectious Diseases, edited by Gerald L. Mandell, R. Gordon Douglas, Jr, and John E. Bennet, 2446 pp, with illus, $185, New York, NY, Churchill Livingstone Inc, 1990.

Principles and Practice of Infectious Diseases has become established as the gold-standard textbook for discussion of infectious diseases. The third edition is expanded and extensively rewritten to reflect new developments in infectious diseases over the last 5 years. Those familiar with previous editions will recall that the text is organized into four sections: "Basic Principles in the Diagnosis and Management of Infectious Diseases," "Major Clinical Syndromes," "Infectious Diseases and Their Etiologic Agents" (the major portion of the text), and "Special Problems." Chapters that have not been rewritten are revised and updated. The star-studded cast of distinguished authors is again present. There are also contributions by new authors.

Important new chapters in the basic principles section are "A Molecular Perspective of Microbial Pathogenicity," "Evaluation of the Patient With Suspected Immunodeficiency," "Mechanisms of Antibiotic Resistance," β-lactam Allergy," and "Quinolones." New contributions to the major clinical syndromes section include "The Acutely Ill Patient With Fever and Rash," "Cystic Fibrosis," "Infections With Prostheses in Bones and Joints," "Slow Infections of the Central Nervous System," and a new subsection, "Acquired Immune Deficiency Syndrome." Pathogens newly recognized since the second edition are discussed in the section on etiologic agents, including papillomaviruses, reoviruses, orbiviruses, parvoviruses, clinically important retroviruses, prions, infection with *Chlamydia pneumoniae,* Lyme disease, borreliosis, the cat-scratch disease agent, microsporidia, and human Ehrlichiosis. The special problems section has been expanded to include a discussion of human immunodeficiency virus type 1 in the health care setting and specific coverage of infections in selected populations, such as bone marrow transplant recipients, solid-organ transplant recipients, the elderly, and patients with spinal cord injuries.

This is the best edition yet of this excellent encyclopedic textbook of infectious diseases. It is a valuable resource for all practitioners, not to mention pathologists, clinical microbiologists, hospital infection-control personnel, residents, fellows, and medical students, who diagnose and treat patients with infectious diseases. The only drawback of this book is its relatively high cost, which may be prohibitive for many.

JOHN A. BRYAN, MD
Atlanta, Ga

Progress in AIDS Pathology, edited by Heidrun Rotterdam, Sheldon C. Sommers, Paul Rácz, and Paul R. Meyer, 249 pp, with illus, $75, New York, NY: Field & Wood Medical Publishers Inc, 1989.

In the preface to *Progress in AIDS Pathology,* it is stated that it "offers a forum to diagnostic pathologists and researchers dealing with (the acquired immunodeficiency syndrome [AIDS]) to discuss their findings and thoughts" with the aim of providing up-to-date information for practicing pathologists. The present volume is a compilation of 17 chapters dealing with a variety of topics and contributions from 48 authors, including many distinguished pathologists and other clinicians and investigators.

The above-mentioned task is done with an excellent introductory chapter surveying the pathologic characteristics of AIDS. Diseases of the central nervous system seen in the course of human immunodeficiency virus (HIV) infection are emphasized in this volume. Chapter 8, dealing with a comparison of pediatric and adult neuropathology, succeeds in meeting the exceptionally high standard of clear medical writing set forth in chapter 1. Because of the increasing awareness of the neuropsychiatric complications of HIV infection, the emphasis given to the nervous system in this book is certainly warranted. The review of lung biopsy specimen interpretation and pulmonary pathology of AIDS (chapter 4) is excellent, providing a remarkable wealth of information in a succinct manner. However, the chapter entitled "AIDS and the Heart" is too brief to address the increasingly apparent significance of cardiac disease in AIDS. Several chapters also report the results of current research. Most of these chapters discuss topics that are either directly relevant to pathologic diagnosis or basic investigation of HIV infection. Many readers of this volume, however, will question the allocation of two chapters (chapters 9 and 10) to reports that deal, respectively, with autopsy findings of three patients with cytomegalovirus infection of the peripheral nervous system and clinically observed motor disorders. The rather speculative chapter dealing with a hypothesis concerning the pathogenesis of HIV infection of the central nervous system is subject to the same criticism.

A strong point of this volume is the bibliography of most chapters. The quality of the illustrations in this book is generally superior, as is the paper used. There are remarkably few typographical errors.

This book appears to have succeeded in its stated goals quite well. *Progress in AIDS Pathology* is likely to prove to be a valuable addition to the libraries of practicing pathologists and those in metropolitan medical centers who have been, unfortunately, forced to deal with a large number of surgical and autopsy specimens from HIV-infected persons.

AZORIDES RAFAEL MORALES, MD
Miami, Fla

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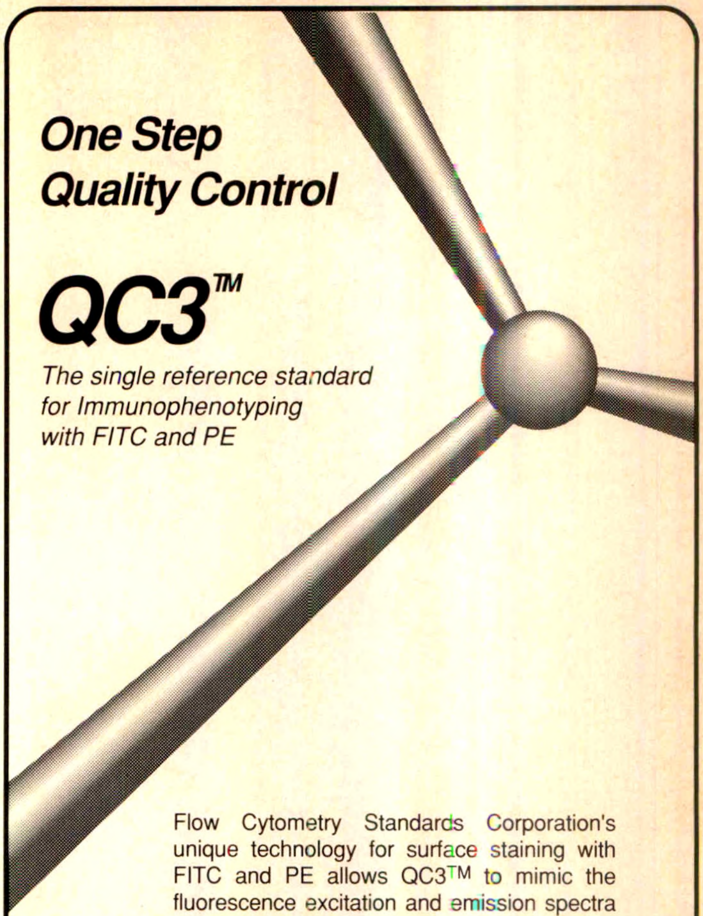

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Abbott introduces ƏHIVAG-1 for the detection of HIV antigen(s)

NOW YOUR LABORATORY CAN ASSIST PHYSICIANS IN...

...Diagnosis
of HIV infection

HIV antigen is detectable as early as two weeks after infection and typically declines at seroconversion.

Seroconversion–when HIV antibodies have risen to detectable levels–usually occurs several weeks up to months after HIV infection.

The period between HIV infection and seroconversion is often associated with an acute illness. Whether or not the individual has flu-like symptoms, the appearance of circulating HIV antigen typcially occurs before IgG antibodies develop.[1,2]

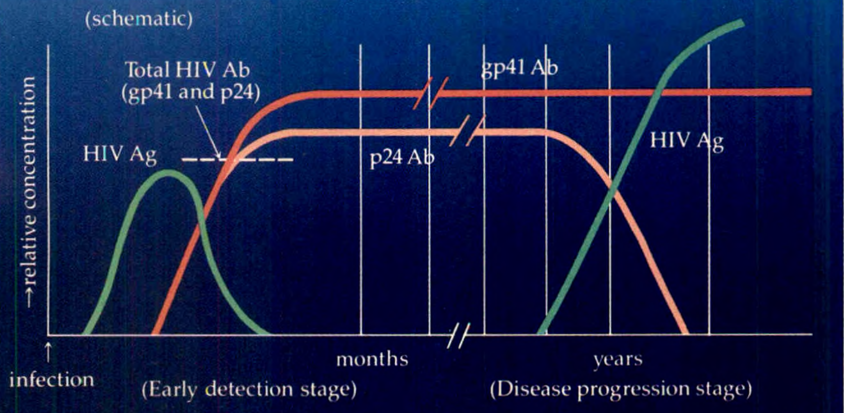
...Prognosis
of HIV disease

HIV antigen typically disappears following seroconversion but reappears in the latter stages of the disease. The reappearance of antigen usually indicates impending clinical deterioration. An asymptomatic HIV-antigen-positive person is six times more likely to develop AIDS within three years than a similar individual who is HIV antigen negative.[3] Thus, testing for the presence of HIV antigen assists physicians in monitoring the progression of the disease.

...Monitoring
of antiviral therapies for clinical research

Antiviral therapies that fight HIV disease can delay the onset of symptoms and help prolong life. Since antigen levels may reflect the level of viral replication, a decline or disappearance of HIV antigen may indicate that antiviral treatment is effective.[4,5] This allows physicians to monitor the action of antiviral drug therapies in research protocols.

The typical serological pattern in HIV infection



HIV antigen may appear as early as two weeks postinfection, but declines and disappears when HIV antibody is produced at seroconversion. HIV antigen then reappears in the latter stages of disease.

ƏHIVAG-1, for *in vitro* diagnostic use, is one of the many Abbott EIA-compatible assays that offer sensitive, specific, and reproducible results. For more information, please write to ABBOTT HIV INFORMATION CENTER, Abbott Diagnostics, D-49B/AP&LM, a Division of Abbott Laboratories, Abbott Park, Illinois 60064.

ƏHIVAG-1

Part of Abbott's broadening line of AIDS diagnostics

References

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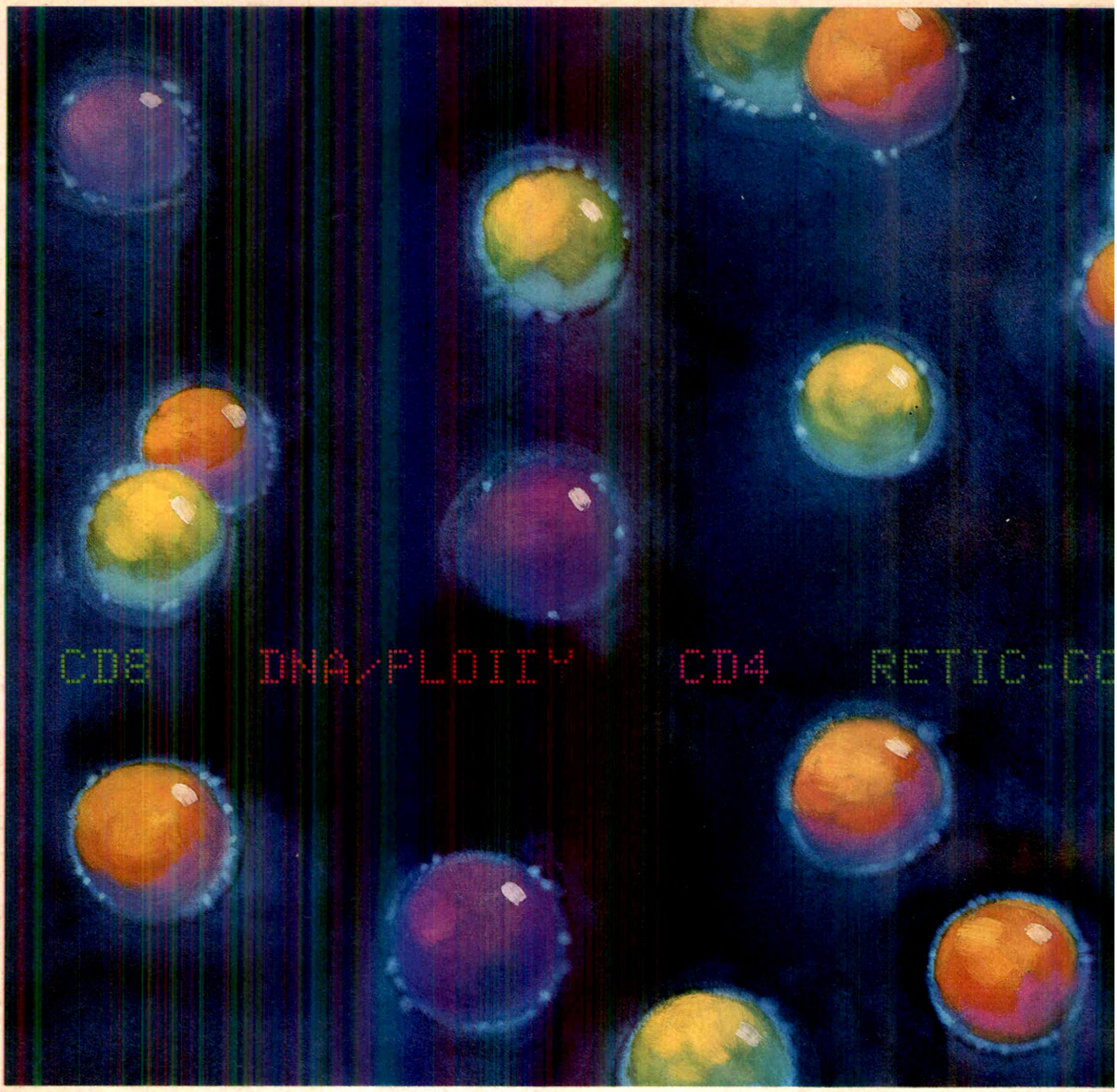

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